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(54) **HIGH AMYLOSE WHEAT**

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CPC ***A23L 1/1025*** (2013.01); ***A23L 1/10*** (2013.01); ***A23L 1/172*** (2013.01); ***C12N 9/107*** (2013.01); ***C12N 15/8245*** (2013.01); ***C12Q 1/48*** (2013.01); *G01N 2333/91131* (2013.01); ***C08B 30/046*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Provided is a Wheat grain (*Triticum aestivum*) comprising an embryo, starch and one, two or three SBEIIa proteins, said embryo comprising two identical alleles of an SBEIIa-A gene, two identical alleles of an SBEIIa-B gene and two identical alleles of an SBEIIa-D gene, wherein the starch has an amylose content of at least 50% (w/w) as a proportion of the extractable starch of the grain, and wherein at least one of the SBEIIa proteins is produced in the developing wheat endosperm and has starch branching enzyme activity.

30 Claims, 21 Drawing Sheets

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FIGURE 1

FIGURE 1 (CONTINUED)

FIGURE 2

FIGURE 3

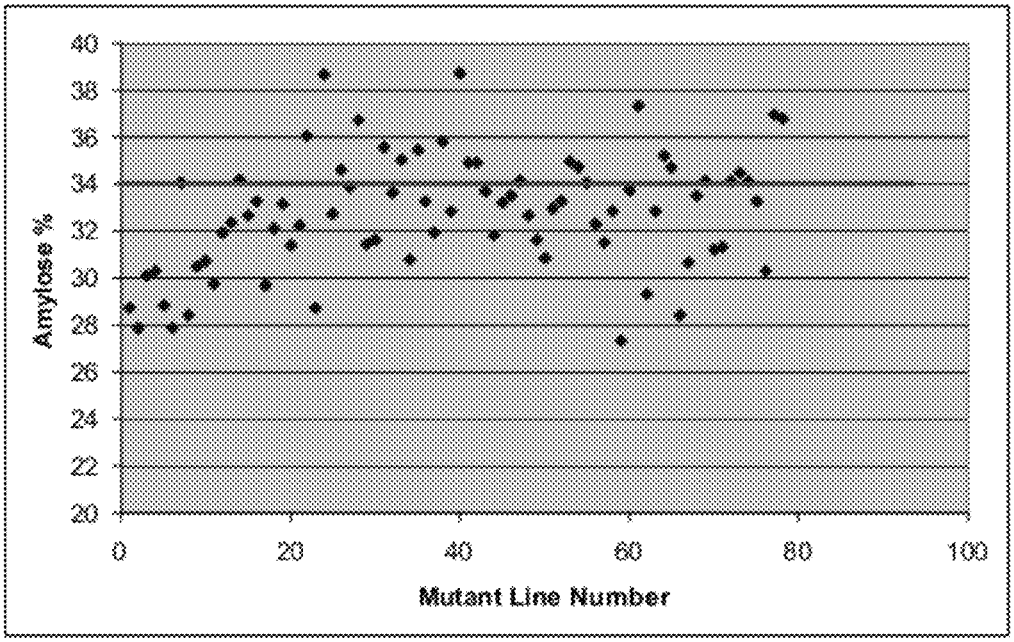

Amylose %
40
38
36
34
32
30
28
26
24
22
20
0
20
40
60
80
100
Mutant Line Number

FIGURE 4

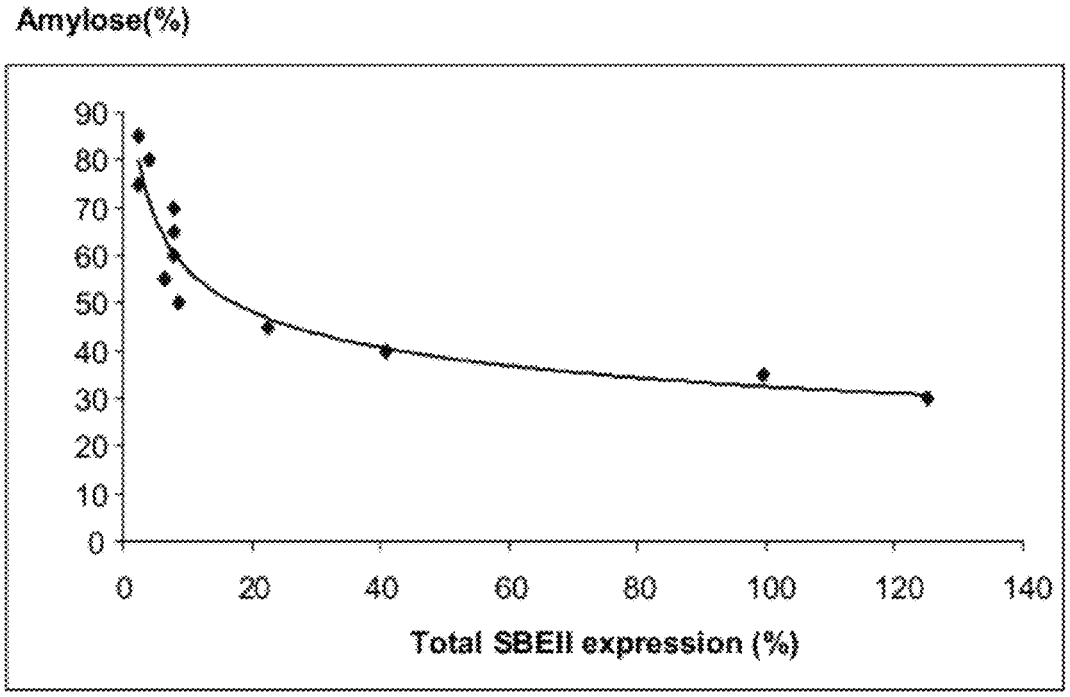

Amylose(%)
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Total SBEII expression (%)

FIGURE 5

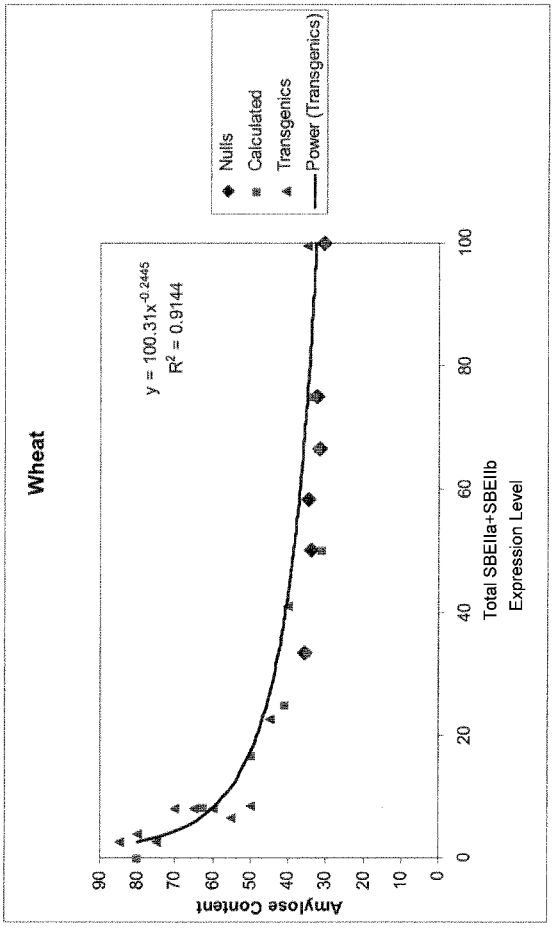

Wheat
Nulls
Calculated
Transgenics
Power (Transgenics)
y = 100.31x^-0.2445
R² = 0.9144
Amylose Content
Total SBEIIa+SBEIIb
Expression Level
0
10
20
30
40
50
60
70
80
90
0
20
40
60
80
100

FIGURE 6

Clade IIa B genome F1-13 0686
Clade IIa A genome_F1-15 0687
Clade IIa D genome F1-1 0678

FIGURE 7

FIGURE 7 (CONTINUED)

FIGURE 8

FIGURE 8 (CONTINUED)

10 20 30 40 50 60 70 80 90 100
GATACCTGAAATTCGACACAACGCCCAACGTGACATCAGCCCGAGTCCAAACCTTCAACTTCTACAAACCGACTCAGCCAATTGTCGAA
CS_exon_3a D genome
CS_exon_3b B genome
CS_exon_3d A genome
110 120 130 140 150 160 170 180 190 200
ACAATCACTGATGTTTACCAAAGGAGTTAAGAACTAGTCGTGGCGAAAACCCCGAGTTTTCCAAACCAGGAATTCGCCAAATATACGAGA
CS_exon_3a D genome
CS_exon_3b B genome
CS_exon_3d A genome
210 220 230 240
TTCAACCAACTCTCAAAGATTTCGAACCATTTGACTCG
CS_exon_3a D genome
CS_exon_3b B genome
CS_exon_3d A genome

FIGURE 9

10 20 30 40 50 60 70 80 90 100
CS_exon 1a A genome
110 120 130 140 150 160 170 180 190 200
CS_exon 1a A genome
210 220 230 240 250 260 270
CS_exon 1a A genome

FIGURE 10

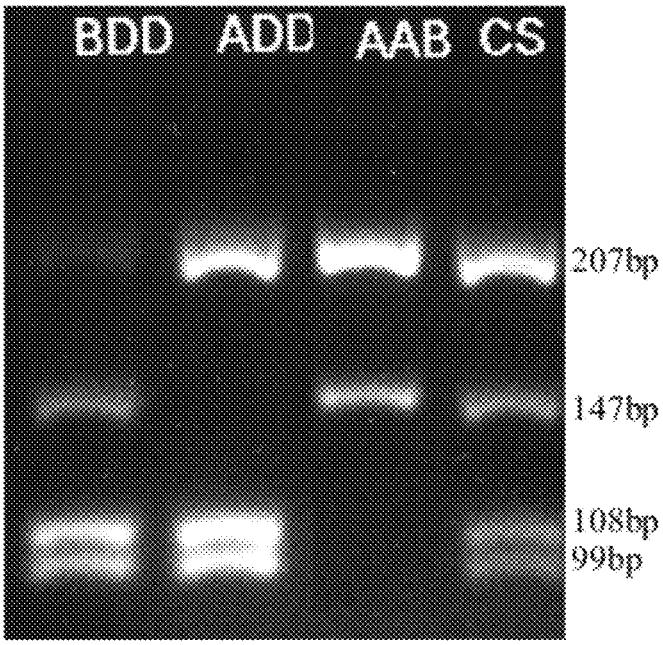


FIGURE 11

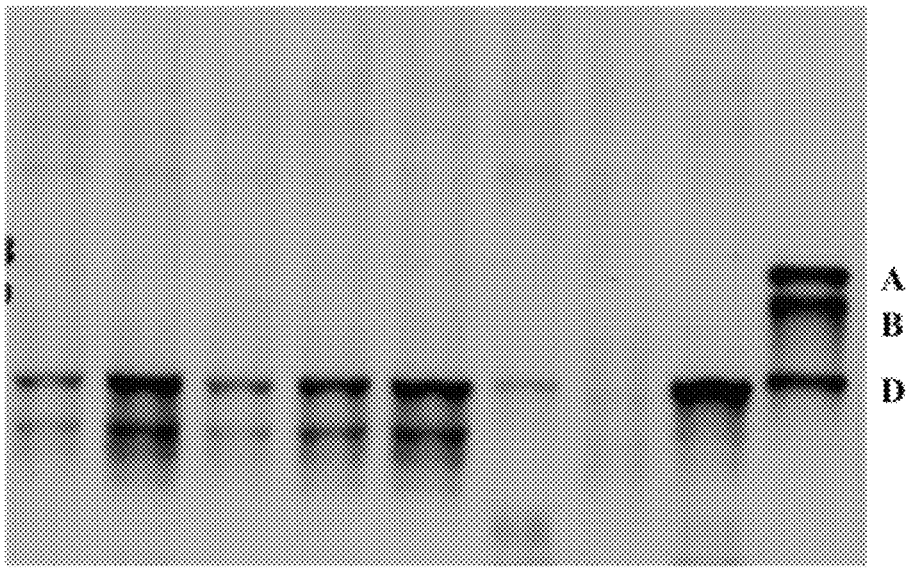


FIGURE 12

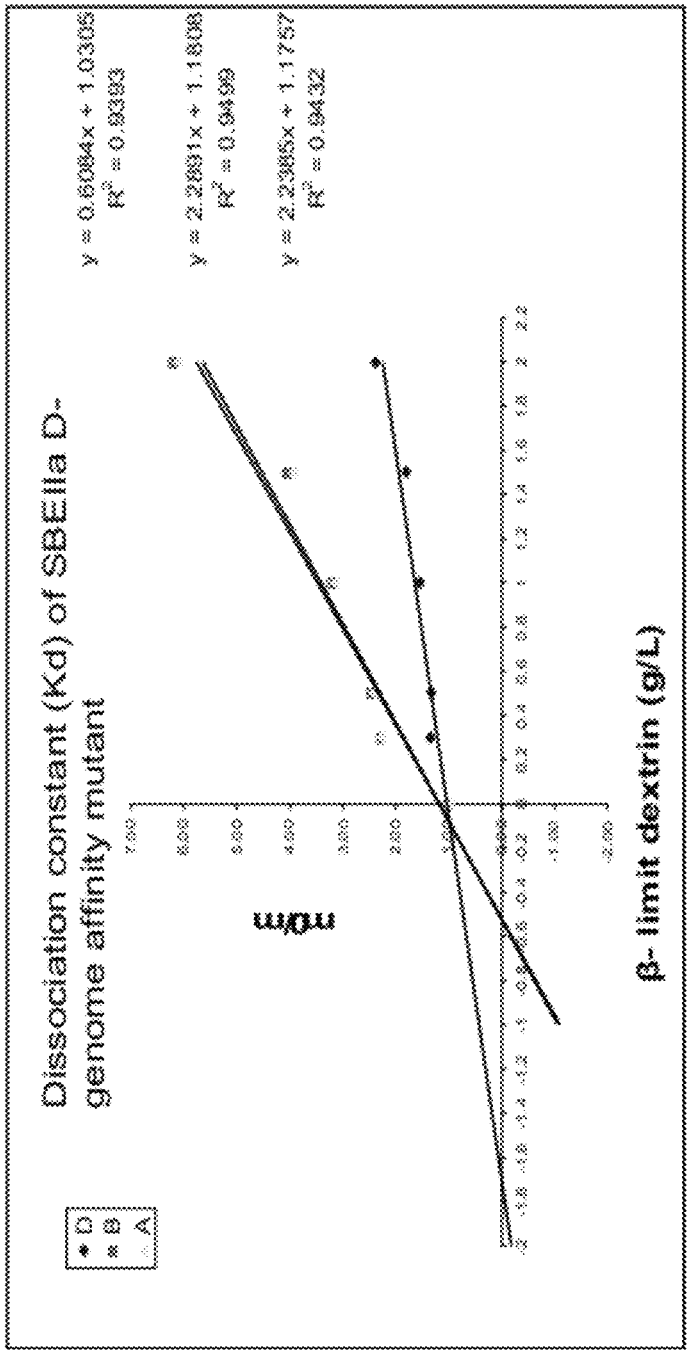

Dissociation constant (Kd) of SBEIIa D-genome affinity mutant
y = 0.6094x + 1.0305
R² = 0.9393
y = 2.2691x + 1.1808
R² = 0.9499
y = 2.3365x + 1.1757
R² = 0.9432
D
B
A
nm
β- limit dextrin (g/L)
Kd: D = 1.69 g/L
B = 0.52 g/L
A = 0.53 g/L

FIGURE 13

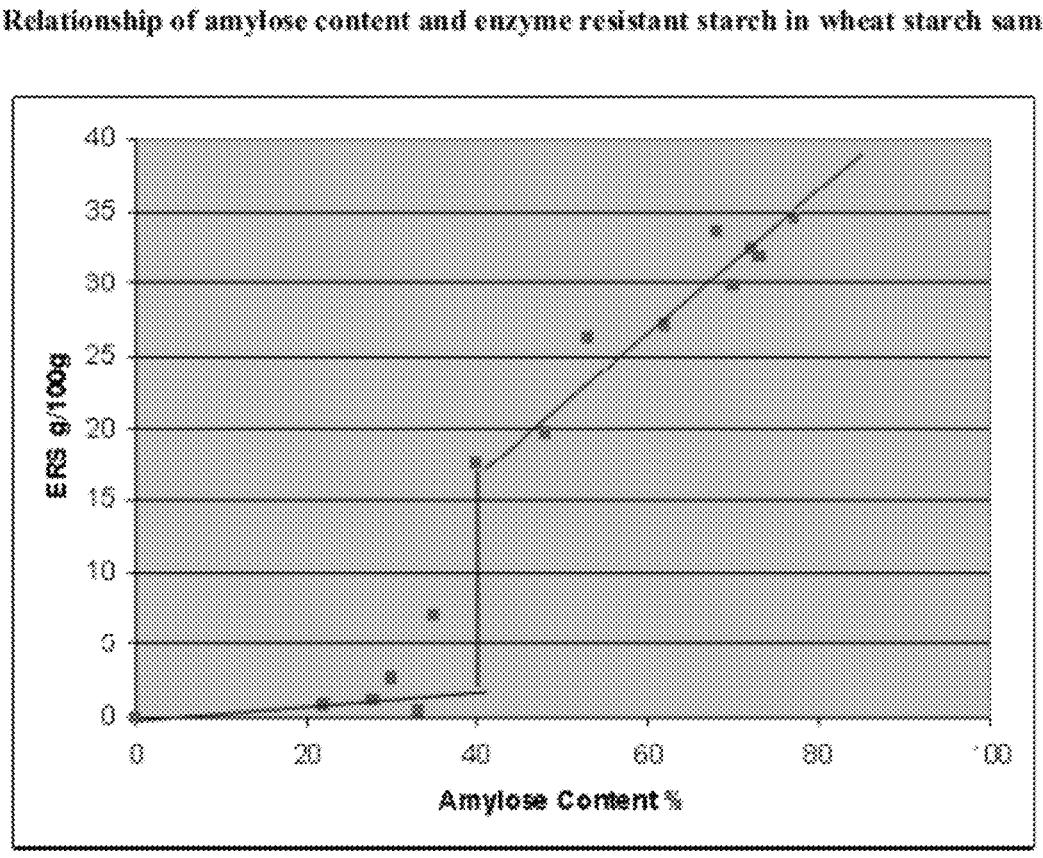

Relationship of amylose content and enzyme resistant starch in wheat starch samples
ERS g/100g
40
35
30
25
20
15
10
0
0
20
40
60
80
Amylose Content %

FIGURE 14

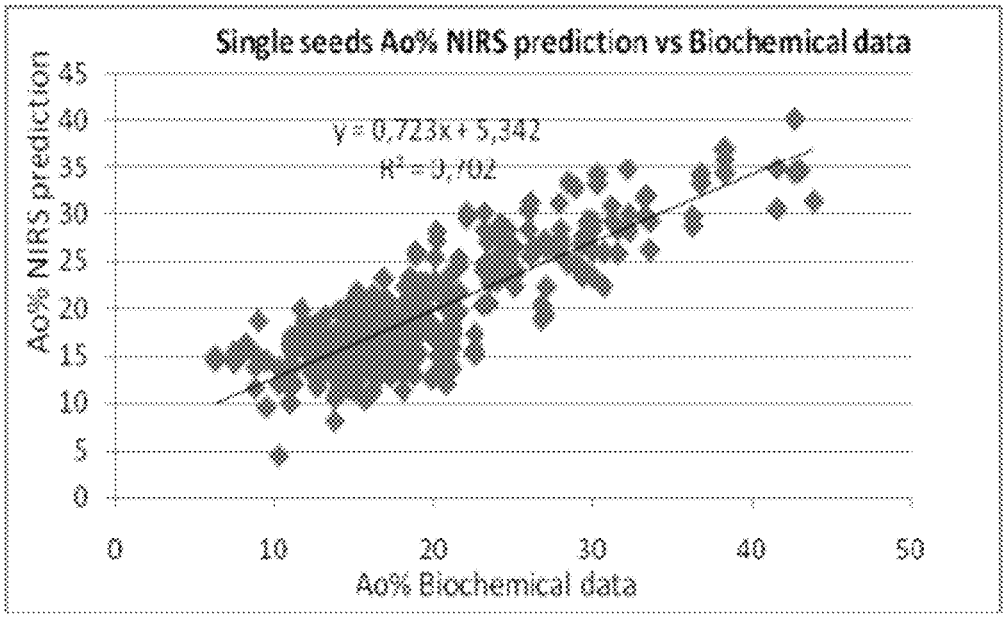

Single seeds Ao% NIRS prediction vs Biochemical data
y = 0,723x + 5,342
R² = 0,702
Ao% NIRS prediction
Ao% Biochemical data
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40
50

FIGURE 15

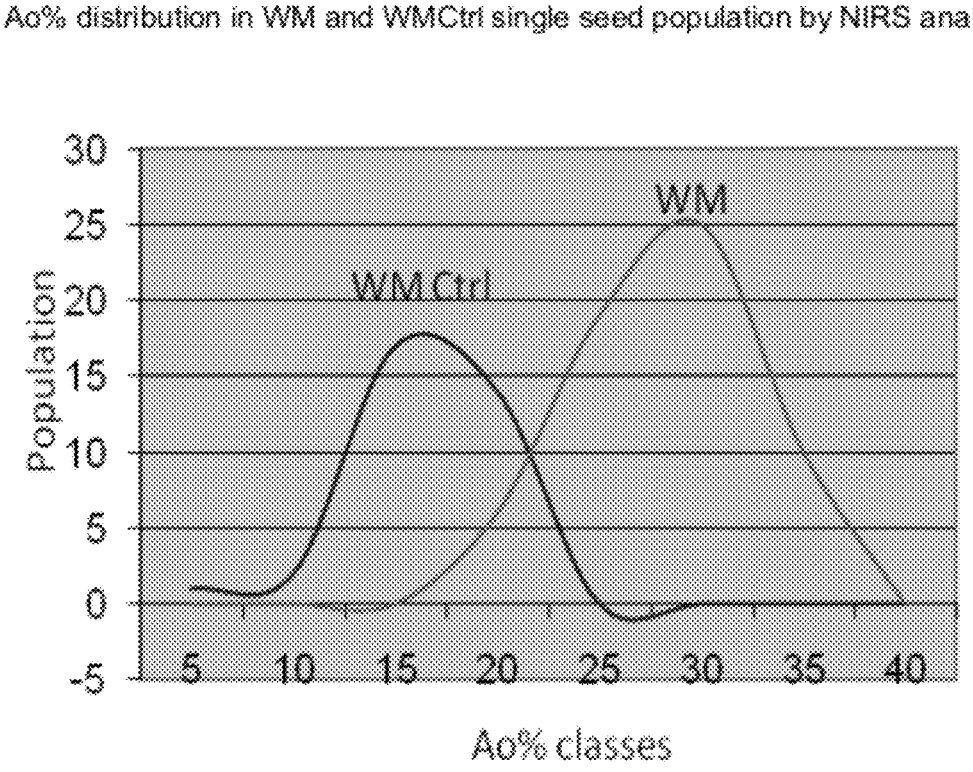
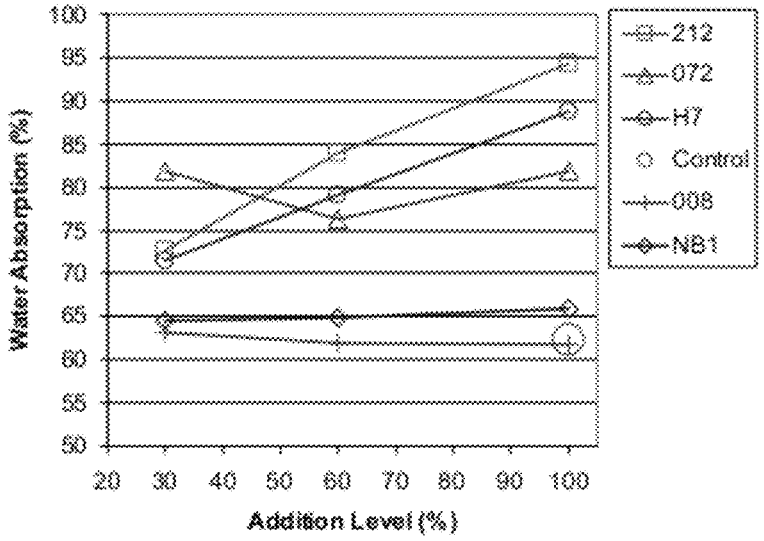

Ao% distribution in WM and WMCtrl single seed population by NIRS analysis
Population
30
25
20
15
10
5
0
-5
5
10
15
20
25
30
35
40
WM
WM Ctrl
Ao% classes

FIGURE 16

(a)


Water Absorption (%)
100
95
90
85
80
75
70
65
60
55
50
20 30 40 50 60 70 80 90 100
Addition Level (%)
212
072
H7
Control
008
NB1

(b)

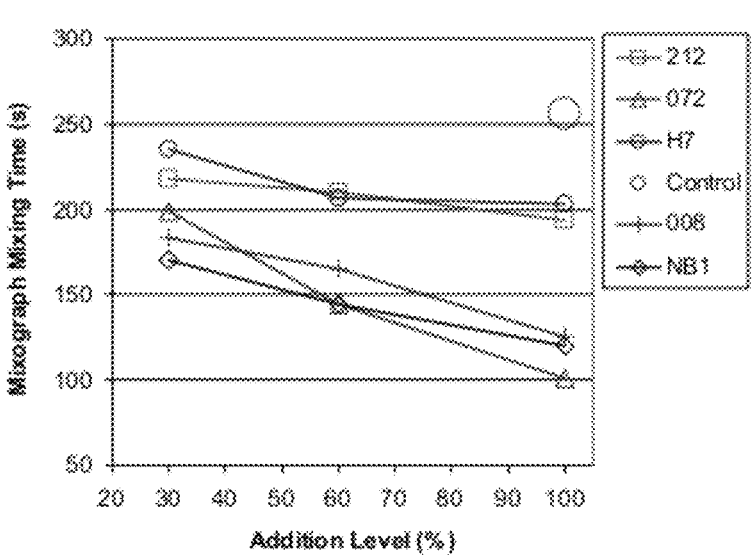
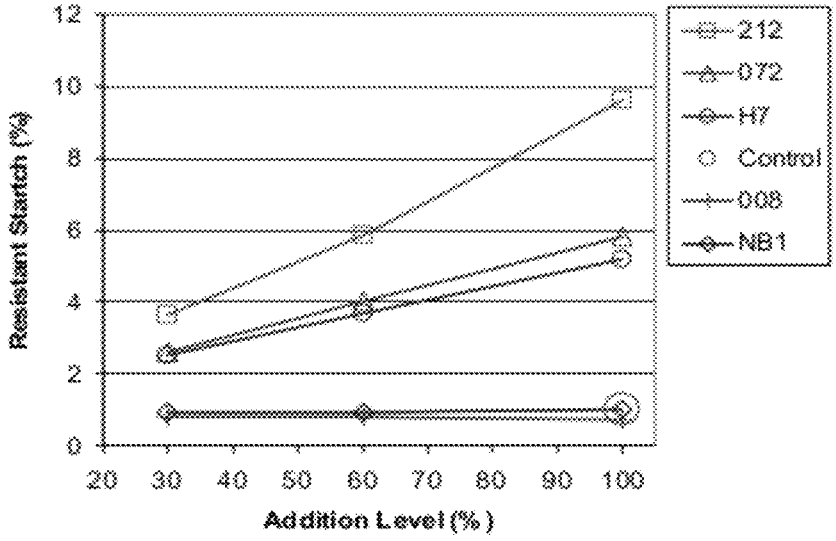

Mixograph Mixing Time (s)
300
250
200
150
100
50
20 30 40 50 60 70 80 90 100
Addition Level (%)
212
072
H7
Control
008
NB1

FIGURE 17

(a)


Resistant Starch (%)
12
10
8
6
4
2
0
20 30 40 50 60 70 80 90 100
Addition Level (%)
212
072
H7
Control
008
NB1

(b)

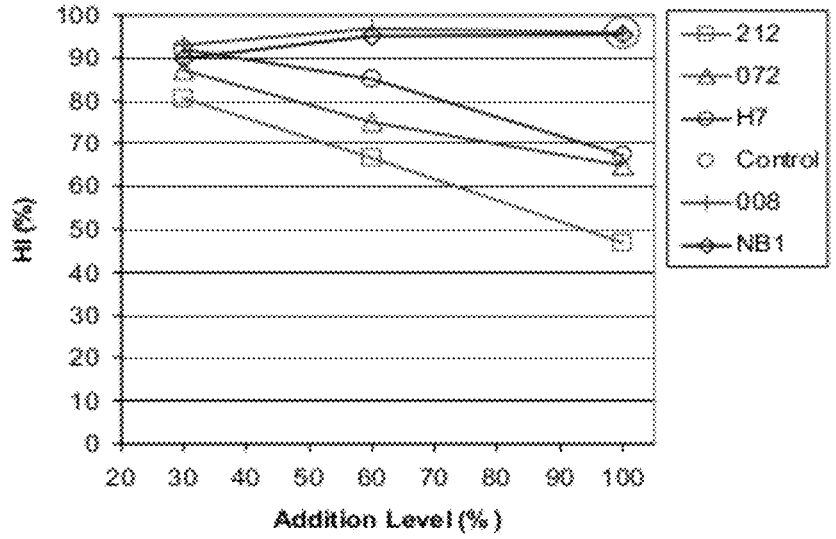

HI (%)
100
90
80
70
60
50
40
30
20
10
0
20 30 40 50 60 70 80 90 100
Addition Level (%)
212
072
H7
Control
008
NB1

FIGURE 18

HIGH AMYLOSE WHEAT

This application claims priority of U.S. Provisional Application No. 61/410,288, filed Nov. 4, 2010, the contents of which are hereby incorporated by reference.

This application incorporates-by-reference nucleotide and/or amino acid sequences which are present in the file named "140912_0687_92306_A_SequenceListing_REB.txt", which is 109 kilobytes in size, and which was created Sep. 12, 2014 in the IBM-PC machine format, having an operating system compatibility with MS-Windows, which is contained in the text file filed Sep. 12, 2014 as part of this application.

Throughout this application, various publications are referenced, including referenced in parenthesis. Full citations for publications referenced in parenthesis may be found listed in alphabetical order at the end of the specification immediately preceding the claims. The disclosures of all referenced publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

FIELD

The specification describes methods of obtaining hexaploid wheat plants having high amylose starch and the use of such plants, and particularly grain or starch therefrom in a range of food and non-food products.

BACKGROUND

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

In the last decade, much has been learnt about the molecular, genetic and cellular events underpinning plant life cycles and plant production. One particularly important plant product is wheat grain. Wheat grain is a staple food in many countries and it supplies at least 20% of the food kilojoules for the total world population. Starch is the major component of wheat grain and is used in a vast range of food and non-food products. Starch characteristics vary and they play a key role in determining the suitability of wheat starch for a particular end use. Despite this huge global consumption and despite an increased awareness of the importance of starch functionality on end product quality, research on genetic variation in wheat and its precise impact on starch characteristics lags behind that for other commercially important plant crops.

Bread wheat (*Triticum aestivum*) is a hexaploid having three pairs of homoeologous chromosomes defining genomes A, B and D. The endosperm of grain comprises 2 haploid complements from a maternal cell and 1 from a paternal cell. The embryo of wheat grain comprises one haploid complement from each of the maternal and paternal cells. Hexaploidy has been considered a significant obstacle in researching and developing useful variants of wheat. In fact, very little is known regarding how homoeologous genes of wheat interact, how their expression is regulated, and how the different proteins produced by homoeologous genes work separately or in concert.

Cereal starch is made up of two glucose polymers, amylose and amylopectin. The ratio of amylose to amylopectin appears to be a major determinant in (i) the health benefit of wheat grain and wheat starch and (ii) the end quality of products comprising wheat starch.

Amylose is an essentially linear polymer of α-1,4 linked glucose units, while amylopectin is highly branched with α-1,6 glucosidic unit bonds linking linear chains.

High amylose starches are of particular interest for their health benefits. Foods comprising high amylose have been found inter alia to be naturally higher in resistant starch, a form of dietary fibre. RS is starch or starch digestive products that are not digested or absorbed in the small intestine. Resistant starch is increasingly seen to have an important role in promoting intestinal health and in protecting against diseases such as colorectal cancer, type II diabetes, obesity, heart disease and osteoporosis. High amylose starches have been developed in certain grains such as maize and barley for use in foods as a means of promoting bowel health. The beneficial effects of resistant starch result from the provision of a nutrient to the large bowel wherein the intestinal microflora are given an energy source which is fermented to form inter alia short chain fatty acids. These short chain fatty acids provide nutrients for the colonocytes, enhance the uptake of certain nutrients across the large bowel and promote physiological activity of the colon. Generally, if resistant starches or other dietary fibre are not provided to the colon it becomes metabolically relatively inactive. Thus high amylose products have the potential to facilitate increased consumption of fibre. Some of the potential health benefits of consuming high amylose wheat grains or their products such as starch include its role in regulating sugar and insulin and lipid levels, promoting intestinal heath, producing food of lower calorie value that promote satiety, improving laxation, water volume of faeces, promoting growth of probiotic bacteria, and enhancing faecal bile acid excretion.

Most processed starchy foods contain very little RS. The breads made using wild-type wheat flour and a conventional formulation and baking process contained <1% RS. In comparison, breads baked using the same process and storage conditions but containing the modified high amylose wheats had levels of RS as much as 10-fold higher (see International Publication No. WO 2006/069422). Legumes, which are one of the few rich sources of RS in the human diet, contain levels of RS that are normally <5%. Therefore, consumption of the high amylose wheat bread in amounts normally consumed by adults (e.g. 200 g/d) would readily supply at least 5-12 g of RS. Thus, incorporation of the high amylose wheat into food products has the potential to make a considerable contribution to dietary RS intakes of developed nations, where average daily intakes of RS are estimated to be only about 5 g.

Starch is widely used in the food, paper and chemical industries. The physical structure of starch can have an important impact on the nutritional and handling properties of starch for food or non-food or industrial products. Certain characteristics can be taken as an indication of starch structure including the distribution of amylopectin chain length, the degree and type of crystallinity, and properties such as gelatinisation temperature, viscosity and swelling volume. Changes in amylopectin chain length may be an indicator of altered crystallinity, gelatinisation or retrogradation of the amylopectin.

Whilst chemically or otherwise modified starches can be used in foods that provide functionality not normally afforded by unmodified sources, such processing has a tendency to either alter other components of value or carry the perception of being undesirable due to processes involved in modification. Therefore it is preferable to provide sources of constituents that can be used in unmodified form in foods.

Starch is initially synthesized in plants in chloroplasts of photosynthesizing tissues such as leaves, in the form of transitory starch. This is mobilized during subsequent dark periods to supply carbon for export to sink organs and energy metabolism, or for storage in organs such as seeds or tubers. Synthesis and long-term storage of starch occurs in the amyloplasts of the storage organs, such as the endosperm, where the starch is deposited as semicrystalline granules up to 100 μm in diameter. Granules contain both amylose and amylopectin, the former typically as amorphous material in the native starch granule while the latter is semicrystalline through stacking of the linear glucosidic chains. Granules also contain some of the proteins involved in starch biosynthesis.

The synthases of starch in the endosperm is carried out in four essential steps. ADP-glucose pyrophosphorylase (ADGP) catalyses the synthesis of ADP-glucose from glucose-1-phosphate and ATP. Starch synthases then promote the transfer of ADP-glucose to the end of an α-1,4 linked glucose unit. Thirdly, starch branching enzymes (SBE) form new α-1,6 linkages in α-polyglucans. Starch debranching enzymes (SDBE) then remove some the branch linkages through a mechanism that has not been fully resolved.

While it is clear that at least these four activities are required for normal starch granule synthesis in higher plants, multiple isoforms of enzymes taking part in one of the four activities are found in the endosperm of higher plants. Specific roles for some isozymes have been proposed on the basis of mutational analysis or through the modification of gene expression levels using transgenic approaches (Abel et al., 1996; Jobling et al., 1999; Schwall et al., 2000). However, the precise contributions of each isoform of each activity to starch biosynthesis are still not known, and these contributions appear to differ markedly between species.

In the cereal endosperm, two isoforms of ADP-glucose pyrophosphorylase (ADGP) are present, one form within the amyloplast, and one form in the cytoplasm. Each form is composed of two subunit types. The shrunken (sh2) and brittle (bt2) mutants in maize represent lesions in large and small subunits respectively.

Some efforts have focussed on starch synthase enzymes to investigate strategies to modulate the amylose/amylopectin ratio in wheat (see Sestili et al. 2010).

Four classes of starch synthase (SS) are found in the cereal endosperm, an isoform exclusively localised within the starch granule (granule-bound starch synthase (GBSS)) two forms that are partitioned between the granule and the soluble fraction (SSI and SSH) and a fourth form that is entirely located in the soluble fraction (SSIII). GBSS has been shown to be essential for amylose synthesis and mutations in SSII and SSIII have been shown to alter amylopectin structure.

A mutant wheat plant entirely lacking the SGP-1 (SSIIa) protein was produced by crossing lines which were lacking the A, 13 and D genome specific forms of SGP-1 (SSII) protein (Yamamori et al., 2000). Examination of the SSII null seeds showed that the mutation resulted in alterations in amylopectin structure, deformed starch granules, and an elevated relative amylose content to about 30-37% of the starch, which was an increase of about 8% over the wild-type level (Yamamori et al., 2000). Amylose was measured by colorimetric measurement, amperometric titration (both for iodine binding) and a concanavalin A method. Starch from the SSII null mutant exhibited a decreased gelatinisation temperature compared to starch from an equivalent, non-mutant plant. Starch content was reduced from 60% in the wild-type to below 50% in the SSII-null grain.

In maize, the dull1 mutation causes decreased starch content and increased amylose levels in endosperm, with the extent of the change depended on the genetic background, and increased degree of branching in the remaining amylopectin. The gene corresponding to the mutation was identified and isolated by a transposon-tagging strategy using the transposon mutator (Mu) and shown to encode the enzyme designated starch synthase II (SSII). The enzyme is now recognized as a member of the SSIII family in cereals. Mutant endosperm had reduced levels of SBEIIa activity associated with the dull1 mutation. It is not known if these findings are relevant to other cereals.

Lines of barley having an elevated proportion of amylose in grain starch have been identified. These include High Amylose Glacier (AC38) which has a relative amylose content of about 45%, and chemically induced mutations in the SSIIa gene of barley which raised levels of amylose in kernel starch to about 65-70% (WO 02/37955 A1; Morell et al., 2003). The starch showed reduced gelatinisation temperatures.

Two main classes of SBEs are known in plants, SBEI and SBEII. SBEII can be further categorized into two types in cereals, SBEIIa and SBEIIb. Additional forms of SBEs are also reported in some cereals, a putative 149 kDa SBEI from wheat and a 50/51 kDa SBE from barley.

Sequence alignment reveals a high degree of sequence similarity at both the nucleotide and amino acid levels and allows the grouping into the SBEI, SBEIIa and SBEIIb classes. SBEIIa and SBEIIb generally exhibit around 80% nucleotide sequence identity to each other, particularly in the central regions of the genes.

In maize and rice, high amylose phenotypes have been shown to result from lesions in the SBEIIb gene, also known as the amylose extender (ae) gene (Boyer and Preiss, 1981, Mizuno et al., 1993; Nishi et al., 2001). In these SBEIIb mutants, endosperm starch grains showed an abnormal morphology, amylose content was significantly elevated, the branch frequency of the residual amylopectin was reduced and the proportion of short chains (<DP17, especially DP8-12) was lower. Moreover, the gelatinisation temperature of the starch was increased. In addition, there was a significant pool of material that was defined as "intermediate" between amylose and amylopectin (Boyer et al., 1980, Takeda et al 1993b). In contrast, maize plants mutant in the SBEIIa gene due to a mutator (Mu) insertional element and consequently lacking SBEIIa protein expression were indistinguishable from wild-type plants in the branching of endosperm starch (Blauth et al., 2001), although they were altered in leaf starch. In both maize and rice, the SBEIIa and SBEIIb genes are not linked in the genome.

SBEIIa, SBEIIb and SBEI may also be distinguished by their expression patterns, both temporal and spatial, in endosperm and in other tissues. SBEI is expressed from mid-endosperm development onwards in wheat and maize (Morell et al., 1997). In contrast, SBEIIa and SBEIIb are expressed from an early stage of endosperm development. In maize, SBEIIb is the predominant form in the endosperm whereas SBEIIa is present at high expression levels in the leaf (Gao et al., 1997). In rice, SBEIIa and SBEIIb are found in the endosperm in approximately equal amounts. However, there are differences in timing and tissues of expression. SBEIIa is expressed at an earlier stage of seed development, being detected at 3 days after flowering, and was expressed in leaves, while SBEIIb was not detectable at 3 days after flowering and was most abundant in developing seeds at 7-10 days after flowering and was not expressed in leaves. In wheat endosperm, SBEI (Morell et al, 1997) is found exclusively in

the soluble fraction, while SBEIIa and SBEIIb are found in both soluble and starch-granule associated fractions (Rahman et al., 1995).

Very high amylose varieties of maize have been known for some time. Low amylopectin starch maize which contains very high amylose content (>90%) was achieved by a considerable reduction in the SBEI activity together with an almost complete inactivation of SBEII activity (Sidebottom et al., 1998).

In potato, down regulation of the main SBE in tubers (SBE B, equivalent to SBEI) by antisense methods resulted in some novel starch characteristics but did not alter the amylose content (Safford et al., 1998). Antisense inhibition of the less abundant form of SBE (SBE A, analogous to SBEII in cereals) resulted in a moderate increase in amylose content to 38% (Jobling et al., 1999). However, the down regulation of both SBEII and SBEI gave much greater increases in the relative amylose content, to 60-89%, than the down-regulation of SBEII alone (Schwall et al., 2000).

International Publication No. WO 2005/001098 and International Publication No. WO 2006/069422 describe inter alia transgenic hexaploid wheat comprising exogenous duplex RNA constructs that reduce expression of SBEIIa and/or SBEIIb in the endosperm. Grain from transgenic lines carried either no SBEIIa and/or SBEIIb protein or reduced protein levels. A loss of SBEIIa protein from endosperm was associated with increased relative amylose levels of more than 50%. A loss of SBEIIb protein levels did not appear to substantially alter the proportion of amylose in grain starch. It was proposed but not established that a SBEIIa and/or SBEIIb triple null mutant substantially lacking expression of SBEIIa and SBEIIb proteins would result in further elevations of amylose levels. However, it was not known or predictable from the prior art how many mutant alleles of SBEIIa and/or SBEIIb would be required to provide high amylose levels of at least 50% as a proportion of the total starch. It was also unknown whether the grain of triple null genotypes would be viable or whether the wheat plants would be fertile.

There is a need in the art for improved high amylose wheat plants and for methods of producing same.

SUMMARY

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a mutation" includes a single mutation, as well as two or more mutations; reference to "a plant" includes one plant, as well as two or more plants; and so forth.

Each embodiment in this specification is to be applied mutatis mutandis to every other embodiment unless expressly stated otherwise.

Genes and other genetic material (e.g. mRNA, constructs etc) are represented in italics and their proteinaceous expression products are represented in non-italicised form. Thus, for example, SBEIIa is an expression product of SBEIIa.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A sequence listing is provided after the claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described.

The present invention provides a range of wheat plants having modified starch characteristics.

In one embodiment, the invention provides wheat grain (*Triticum aestivum*) comprising an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain.

In one embodiment, the invention provides wheat grain comprising an embryo and starch, wherein the embryo comprises two identical alleles of an SBEIIa-A gene, two identical alleles of an SBEIIa-B gene and two identical alleles of an SBEIIa-D gene, wherein each of the SBEIIa genes gives rise to an amount of protein (w/w) or a protein having SBEIIa activity which is lower than the corresponding wild-type gene, and at least one of said genes comprises a point mutation, wherein the starch comprises amylose such that the grain has an amylose content of at least 50% (w/w) as a proportion of the extractable starch of the grain.

In one embodiment, the invention provides wheat grain comprising an embryo, starch and one, two or three SBEIIa proteins, said embryo comprising two identical alleles of an SBEIIa-A gene, two identical alleles of an SBEIIa-B gene and two identical alleles of an SBEIIa-D gene, wherein the starch has an amylose content of at least 50% (w/w) as a proportion of the extractable starch of the grain, and wherein at least one of the SBEIIa proteins is produced in the developing wheat endosperm and has starch branching enzyme activity.

In some embodiments, the amount and activity of the SBEIIa protein are reduced. Thus, for example, a grain of the invention may comprise a reduced amount of SBEIIa protein (w/w) which has reduced SBEIIa activity.

In various embodiments, the level or activity of total SBEII or SBEIIa protein in the grain is less than 2% or 2% to 15%, or 3% to 10%, or 2% to 20% or 2% to 25% of the level or activity of total SBEII or SBEIIa protein in the wild-type grain.

In some embodiments, the amount or activity of the SBEIIa protein in the grain is less than 2% of the amount or activity of SBEIIa protein in a wild-type wheat grain.

In another aspect, the grain is from hexaploid wheat.

In one embodiment, the grain is from hexaploid wheat and comprises an embryo, wherein the embryo comprises a loss of function mutation in each of 5 to 12 alleles of endogenous SBEII genes selected from the group consisting of SBEIIa-A, SBEIIa-B, SBEIIa-D, SBEIIb-A, SBEIIb-B and SBEIIb-D. In one particular, said 5 to 12 alleles including 4, 5 or 6 SBEIIa alleles each comprise a loss of function mutation. In another particular, when the number of SBEIIa alleles comprising a loss of function mutation is only 4 then the number of SBEIIb alleles comprising a loss of function mutation is 6. In another embodiment, when the number of SBEIIa alleles comprising a loss of function mutation is 6 then at least two SBEIIb alleles comprise a partial loss of function mutation. In a further embodiment, the hexaploid wheat embryo has no null alleles of SBEIIb genes, or only 1, only 2, only 3, only 4, only 5 or 6 null alleles of SBEIIb genes.

In a further embodiment, the hexaploid wheat embryo has only 2, only 3, only 4 or only 5 null alleles of SBEIIa genes.

In some embodiments, the hexaploid wheat embryo has 6 null alleles of SBEIIa genes.

In some embodiments, the grain or embryo has only 1 null SBEIIa gene.

In some embodiments, the grain or embryo has only 2 null SBEIIa genes. In a further embodiment, the hexaploid wheat embryo has no null alleles of SBEIIb genes, or only 1, only 2, only 3, only 4, only 5 or 6 null alleles of SBEIIb genes.

In yet another embodiment, the null alleles of the SBEIIa or SBEIIb genes are on the A genome, B genome, D genome, A and B genomes, A and D genomes, A and D genomes, or all three of the A, B and D genomes.

In yet another embodiment, the hexaploid wheat embryo comprises 0, 1, 2, 3, 4, 5, or 6 partial loss of function alleles of SBEIIa genes. In some cases the partial loss of function allele of the SBEIIa gene is on the A genome, B genome, D genome, A and B genomes, A and D genomes, A and D genomes, or all three of the A, B and D genomes.

Additionally, in some embodiments, the hexaploid wheat embryo comprises 0, 1, 2, 3, 4, 5, or 6 partial loss of function alleles of SBEIIb genes. In some cases the partial loss of function allele of the SBEIIb gene is on the A genome, B genome, D genome, A and B genomes, A and D genomes, A and D genomes, or all three of the A, B and D genomes.

In other embodiments, the partial loss of function alleles of the SBEIIa or SBEIIb genes are on the A genome, B genome, D genome, A and B genomes, A and D genomes, A and D genomes, or all three of the A, B and D genomes.

In another embodiment, the hexapoloid wheat embryo comprises 5 SBEIIa alleles each comprising a null or partial loss of function mutation and 1 SBEIIa allele which is wild-type.

In another embodiment, the grain is from tetraploid wheat.

In another embodiment, the present invention provides wheat grain from tetrapoloid wheat wherein the grain comprises an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain.

In some embodiments, wherein the embryo comprises a loss of function mutation in each of 5 to 8 alleles of endogenous SBEII genes selected from the group consisting of SBEIIa-A, SBEIIa-B, SBEIIb-A and SBEIIb-B, said 5 to 8 alleles including 2, 3 or 4 SBEIIa alleles each comprising a loss of function mutation, and wherein when the number of SBEIIa alleles comprising a loss of function mutation is only 2 then the number of SBEIIb alleles comprising a loss of function mutation is 4, and when the number of SBEIIa alleles comprising a loss of function mutation is 4 then at least one, preferably at least two such alleles comprise a partial loss of function mutation.

In some embodiments, the embryo has only 2, or only 3, null alleles of SBEIIa genes.

In one particular embodiment, the tetraploid wheat embryo has no null alleles of SBEIIb genes, or only 1, only 2, only 3, or 4, null alleles of SBEIIb genes.

In some embodiments, the one SBEIIb protein is encoded by the A genome, the B, genome or D genome, or the two SBEIIb proteins are encoded by the A and B genomes, A and D, genomes, or B and D genomes.

In some embodiments, the null mutation is independently selected from the group consisting of a deletion mutation, an insertion mutation, a splice-site mutation, a premature translation termination mutation, and a frameshift mutation.

In some embodiments, the null alleles of the SBEIIa or SBEIIb genes are on the A genome, B genome, or both of the A and B genomes.

In other embodiments, the tetraploid wheat embryo comprises 0, 1, 2, 3 or 4, 5, or 6 partial loss of function alleles of SBEIIa genes.

In other embodiments, the embryo comprises 0, 1, 2, 3 or 4 partial loss of function alleles of SBEIIb genes.

In yet another embodiment, the partial loss of function alleles of the SBEIIa or SBEIIb genes are on the A genome, B genome, or both of the A and B genomes.

In some embodiments, the embryo is homozygous for mutant alleles in each of 2 or 3 SBEIIa genes and/or each of 2 or 3 SBEIIb genes.

In other embodiments, the embryo is heterozygous for each of 2 or 3 SBEIIa genes and/or each of 2 or 3 SBEIIb genes.

Usefully, in various embodiments of the present invention the grain comprises both null alleles and partial loss of function alleles of SBEIIa and/or SBEIIb, wherein each of the null alleles is located on a different genome than each of the partial loss of function alleles.

In some embodiments relating to the null alleles, each null mutation is independently selected from the group consisting of a deletion mutation, an insertion mutation, a splice-site mutation, a premature translation termination mutation, and a frameshift mutation. In an embodiment, one or more of the null mutations are non-conservative amino acid substitution mutations or a null mutation has a combination of two or more non-conservative amino acid substitutions. In this context, non-conservative amino acid substitutions are as defined herein. The grain may comprise mutations in each of two SBEIIa genes, each of which are null mutations, and an amino acid substitution mutation in a third SBEIIa gene, wherein each of the null mutations are preferably premature translation termination mutations or deletion mutations, or one premature translation termination mutation and one deletion mutation, and the amino acid substitution mutation is either a conservative amino acid substitution or preferably a non-conservative amino acid substitution.

In some broad embodiments, the grain of the present invention includes one or more null mutations or partial loss of function mutations which are amino acid substitution mutations, which are independently non-conservative or conservative amino acid substitutions.

In some embodiments, the grain of the present invention comprises one point mutation, which is an amino acid substitution mutation.

In some embodiments of the invention, one of the SBEIIa-A, SBEIIa-B or SBEIIa-D genes comprises a point mutation such that the protein encoded by said gene lacks starch branching enzyme activity.

In some embodiments, the grain of the present invention has null alleles which are deletion mutations in the B and D genomes which delete at least part of the SBEIIa-B and SBEIIa-D genes, respectively and wherein the SBEIIa-A gene comprises the point mutation; or having null alleles which are deletion mutations in the A and D genomes which delete at least part of the SBEIIa-A and SBEIIa-D genes, respectively and wherein the SBEIIa-B gene comprises the point mutation; or having null alleles which are deletion mutations in the A and B genomes which delete at least part of the SBEIIa-A and SBEIIa-B genes, respectively and wherein the SBEIIa-D gene comprises the point mutation.

In some embodiments of the invention, the embryo comprises 6 SBEIIb alleles of which at least one has a loss of function mutation.

In some embodiments of the invention, the embryo has no null alleles of SBEIIb genes, or only 2, only 4 or 6 null alleles of SBEIIb genes.

In some embodiments, the grain comprises a null mutation which is a deletion mutation in the A, B or D genome, which deletes at least part of an SBEIIa gene and at least a part of an SBEIIb gene, preferably which deletes the whole of the SBEIIa gene, and/or the SBEIIb gene.

In some embodiments, the grain of the invention comprises a null mutation which is a deletion mutation in the B genome which deletes at least part of the SBEIIa-B gene and at least a part of the SBEIIb-B gene, preferably which deletes the whole of the SBEIIa-B gene and/or the SBEIIb-B gene; or comprising a null mutation which is a deletion mutation in the D genome which deletes at least part of the SBEIIa-D gene and at least a part of an SBEIIb-D gene, preferably which deletes the whole of the SBEIIa-D gene and/or the SBEIIb-D gene; or comprising a null mutation which is a deletion mutation in the B genome which deletes at least part of the SBEIIa-A gene and at least a part of the SBEIIb-A gene, preferably which deletes the whole of the SBEIIa-A gene and/or the SBEIIb-A gene.

In illustrative examples, grain is provided wherein the alleles comprising a partial loss of function mutation each express an SBEIIa or SBEIIb enzyme which in amount and/or activity corresponds to 2% to 60%, or 10% to 50%, of the amount or activity of the corresponding wild-type allele.

In some embodiments, the grain comprises at least one SBEIIa protein which has starch branching activity when expressed in developing endosperm, the protein being present in an amount or having starch branching enzyme activity of between 2% to 60%, or between 10% to 50%, or between 2% to 30%, or between 2% to 15%, or between 3% to 10%, or between 2% to 20% or between 2% to 25% of the amount or activity of the corresponding protein in a wild-type wheat grain.

In some embodiments of the invention, the amount or activity of total SBEII protein in the grain is less than 60%, preferably less than 2%, of the amount or activity of total SBEII protein in a wild-type wheat grain.

In some embodiments of the invention, there is no SBEIIa protein activity in the grain.

Specifically, in some embodiments, the grain is non-transgenic i.e. does not comprise any transgene, or in a more specific embodiment does not comprise an exogenous nucleic acid that encodes an RNA which reduces expression of an SBEIIa gene i.e if it comprises a transgene, that transgene encodes an RNA other than an RNA which reduces expression of an SBEIIa gene. Such RNAs include RNAs which encode proteins that confer herbicide tolerance, disease tolerance, increase nutrient usage efficiency, or drought or other stress tolerance, for example.

In some embodiments, the grain has only one SBEIIa protein as determined by Western blot analysis, and wherein the protein is encoded by one of the SBEIIa-A, SBEIIa-B and SBEIIa-D genes and has reduced starch branching enzyme activity when produced in developing endosperm when compared to an SBEIIa protein encoded by the corresponding wild-type gene.

In some embodiments, the SBEIIa protein has an altered mobility relative to its corresponding wild-type SBEIIa protein, as determined by affinity gel electrophoresis on gels containing starch.

In some embodiments, the grain lacks detectable SBEIIa protein as determined by Western blot analysis.

In some embodiments, the embryo comprises only one or only two SBEIIb proteins which have starch branching enzyme activity when produced in developing endosperm, or only one or only two SBEIIb proteins which are detectable by Western blot analysis.

In relation to loss of function mutations, in some embodiments, at least one, more than one, or all of the loss of function mutations are i) introduced mutations, ii) were induced in a parental wheat plant or seed by mutagenesis with a mutagenic agent such as a chemical agent, biological agent or irradiation, or iii) were introduced in order to modify the plant genome.

In another illustrative embodiment, the grain comprises an exogenous nucleic acid which encodes an RNA which reduces expression of an SBEIIa gene, an SBEIIb gene, or both.

As determined herein, grain is provided in some particular embodiments wherein the grain has a germination rate of about 70% to about 90%, or about 90% to about 100% relative to the germination rate of a control or wild type grain under standard conditions. The standard conditions are preferably as defined herein.

In one particular embodiment, the SBEII activity or SBEIIa activity is determined by assaying the enzymatic activity in grain while it is developing in a wheat plant, or by assaying the amount of SBEII protein such as SBEIIa protein in harvested grain by immunological or other means.

In another aspect, the present invention provides grain, wherein the starch of the grain is at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) amylose as a proportion of the total starch and is characterised by one or more of:

(i) comprising 2% to 30% of the amount of SBEII or SBEIIa relative to wild-type wheat starch granules or starch;
(ii) comprising at least 2% resistant starch;
(iii) comprising a low relative glycaemic index (GI);
(iv) comprising low relative amylopectin levels;
(v) distorted starch granules;
(vi) reduced granule birefringence;
(vii) reduced swelling volume;
(viii) modified chain length distribution and/or branching frequency;
(ix) delayed end of gelatinisation temperature and higher peak temperature;
(x) reduced viscosity (peak viscosity, pasting temperature, etc.);
(xi) increased molecular weight of amylopectin; and/or
(xii) modified % crystallinity % A-type or B-type starch, relative to a wild-type wheat starch granules or starch.

In some embodiments, the grain is comprised in a wheat plant.

In other embodiments, the grain is developing grain, or mature, harvested grain. Preferably the quantity of grain is at least 1 kg weight, or at least 1 tonne weight.

Conveniently, the grain is processed so that it is no longer capable of germinating, such as kibbled, cracked, par-boiled, rolled, pearled, milled or ground grain.

In another aspect the present invention provides a wheat plant which is capable of producing the grain as defined herein including grain comprising an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain

comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain.

In one particular, the wheat plant is both male and female fertile.

In one embodiment, the wheat plant is bread wheat such as *Triticum aestivum* L. ssp. *aestivum* or durum wheat. In other embodiments, the wheat plant is characterised by one or more features of the grain as described herein, preferably including the numbers and types of SBEIIa and SBEIIb mutations as described herein. All combinations of such features are provided.

In another embodiment, the invention provides wholemeal or flour or another food ingredient such as purified starch produced from the grain as defined herein including grain comprising an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain. The wholemeal, flour or other food ingredient may be refined by fractionation, bleaching, heat treatment to stabilise the ingredient, treated with enzymes or blended with other food ingredients such as wholemeal or flour from a wild-type wheat. The flour is preferably white flour, having specifications as known in the art of baking. In a preferred embodiment, the wholemeal, flour or other food is packaged ready for sale as a food ingredient, which package may include instructions of recipes for its use.

The present invention further contemplates wheat starch granules or wheat starch produced from the subject grain. In some embodiments, the starch granules or wheat starch comprise at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) amylose as a proportion of the starch, and are further characterised by one of more of the features:

(i) comprising 2% to 30% of the amount of SBEII or SBEIIa relative to wild-type wheat starch granules or starch;
(ii) comprising at least 2% resistant starch;
(iii) comprising a low relative glycaemic index (GI);
(iv) comprising low relative amylopectin levels;
(v) distorted starch granules;
(vi) reduced granule birefringence;
(vii) reduced swelling volume;
(viii) modified chain length distribution and/or branching frequency;
(ix) delayed end of gelatinisation temperature and higher peak temperature;
(x) reduced viscosity (peak viscosity, pasting temperature, etc.);
(xi) increased molecular weight of amylopectin; and/or
(xii) modified % crystallinity % A-type or B-type starch, relative to a wild-type wheat starch granules or starch.

The present invention further provides a food ingredient that comprises the grain, wholemeal, flour, starch granules, or starch as defined herein, for use in the production of foods, for consumption by non-human animals or preferably humans.

In some embodiments, the food ingredient comprises grain wherein the grain is kibbled, cracked, par-boiled, rolled, pearled, milled or ground grain or any combination of these.

The invention also provides food or drink products which comprises a food or drink ingredient at a level of at least 10% on a dry weight basis, wherein the food ingredient is or comprises the grain, wholemeal, flour, starch granules, or starch as defined herein. Preferably the food or drink product is packaged ready for sale.

In another embodiment, the invention provides a composition or blend comprising the grain, wholemeal, flour, wheat starch granules or wheat starch as defined herein, at a level of at least 10% by weight, and wheat grain having a level of amylose lower than about 50% (w/w) or flour, wholemeal, starch granules or starch obtained therefrom. Preferably, the wheat grain having a level of amylase lower than 50% (w/w) is wild-type wheat grain.

Methods are provided for obtaining or identifying or selecting or producing a wheat plant that produces grain comprising an amylase content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain. The wheat plant may be identified or selected from a population of multiple candidate plants, such as a mutagenised population or a population of plants resulting from a crossing process or a back-crossing/breeding process.

In some embodiments, the method comprises: (i) crossing two parental wheat plants each comprising a loss of function mutation in each of one, two or three SBEIIa or SBEIIb genes selected from the group consisting of SBEIIa-A, SBEIIa-B, SBEIIa-D, SBEIIb-A, SBEIIb-B and SBEIIb-D, or of mutagenising a parental plant comprising said loss of function mutations; and (ii) screening plants or grain obtained from the cross or mutagenesis, or progeny plants or grain obtained therefrom, by analysing DNA. RNA, protein, starch granules or starch from the plants or grain, and (iii) selecting a fertile plant that exhibits a level or activity of SBEII or SBEIIa in its grain that is 2% to 30% the level or activity of the respective protein in a wild-type grain. Alternatively, the method comprises steps (ii) and (iii) above, with step (i) being optional, such as when selecting or identifying a plant from a population of multiple candidate plants.

In some embodiments of the method step (ii) includes screening first, second and/or subsequent generation progeny plants or grain for a loss of function mutation in 5 to 12 alleles of 6 endogenous genes encoding SBEII protein including 4, 5 or 6 SBEIIa alleles, and wherein when the number of mutant SBEIIa alleles is 4 then the number of mutant SBEIIb alleles is 6, and when the number of mutant SBEIIa alleles is 6 then at least two such mutants are partial mutations.

In some embodiments, the grain of the selected fertile wheat plant is characterised by one or more features as defined herein.

The invention further provides methods of obtaining a hexaploid or tetraploid wheat plant that produces grain comprising an amylose content of at least 50% (w/w), or at least 60% (w/w) or at least 67% (w/w) as a proportion of the total starch in the grain. In some embodiments, the method comprises (i) introducing into a wheat cell an exogenous nucleic acid that encodes an RNA which reduces expression of one or more genes encoding total SBEII protein or SBEIIa protein, (ii) regenerating a transgenic wheat plant comprising the exogenous nucleic acid from the cell of step (i), and (iii) screening for and selecting first, second or subsequent generation progeny of the transgenic wheat plant which produce grain having 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type plant. Preferably, the RNA molecule is a double-stranded RNA molecule or a micro-RNA precursor molecule, which is preferably expressed from a chimeric DNA comprising a DNA region which, when transcribed, produces the RNA molecule, operably linked to a heterologous promoter such as an endosperm-specific promoter. The chimeric DNA may be introduced into a wheat cell which comprises one or more SBEIIa or SBEIIb mutations,

such that the total SBEII activity is reduced in the transgenic plant by a combination of mutation(s) and inhibitory RNA molecule(s).

In some embodiments, grain having 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type plant is indicative that at least 3 SBEIIa genes or 2 SBEIIa genes and 3 SBEIIb genes of the plant comprise a loss of function mutation and therefore that grain of the plant comprises more than 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) amylose as a proportion of the total starch in the grain. In some embodiments, the presence of at least a low level of SBEIIa protein is indicative that the plant is fertile.

In another embodiment, the invention provides a method of screening a wheat plant or grain, the method comprising screening a plant or grain for mutations in a SBEIIa gene or SBEIIa and SBEIIb genes in each of A, B and D genomes of hexaploid wheat or the A and B genomes of tetraploid wheat using one or more of the primers selected from the group consisting of SEQ ID NO: 36 to 149.

In another embodiment, the invention provides a method of screening a wheat plant or grain, the method comprising (i) determining the level or activity of SBEIIa and/or SBEIIb relative to the level or activity in a wild type or control plant or grain and selecting plant or grain having 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type plant.

In yet another embodiment, the invention provides a method of producing a food or a drink comprising (i) obtaining grain of the invention, (ii) processing the grain to produce a food or drink ingredient, and (iii) adding food or drink ingredient from another food or drink ingredient, thereby producing the food or drink.

In another aspect, the invention provides a method for improving one or more parameters of metabolic health, bowel health or cardiovascular health in a subject, or of preventing or reducing the severity or incidence of a metabolic disease such as diabetes, bowel disease or cardiovascular disease, comprising providing to the subject the grain, food or drink as defined herein. The invention also provides for the use of the grain, or products derived therefrom, for use in therapy or prophylaxis of the metabolic disease, bowel disease or cardiovascular disease.

Accordingly, similar aspects of the invention provide the subject grain, food or drink for using in for improving one or more parameters of metabolic health, bowel health or cardiovascular health in a subject, or of preventing or reducing the severity or incidence of a metabolic disease such as diabetes, bowel disease or cardiovascular disease.

Accordingly, similar aspects the invention provide for the use of the subject grain, food or drink for improving one or more parameters of metabolic health, bowel health or cardiovascular health in a subject, or of preventing or reducing the severity or incidence of a metabolic disease such as diabetes, bowel disease or cardiovascular disease.

Accordingly, in some embodiments the invention provides the food or drink product as defined herein for use in improving one or more parameters of metabolic health, bowel health or cardiovascular health, or of preventing or reducing the severity or incidence of metabolic, bowel or cardiovascular disease in a subject.

In another embodiment, the invention provides a method of producing grain, comprising the steps of i) obtaining a wheat plant that is capable of producing the grain as defined herein comprising an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain and, ii) harvesting wheat grain from the plant, and iii) optionally, processing the grain.

In another embodiment, the invention provides a method of producing starch, comprising the steps of i) obtaining wheat grain as defined herein including comprising an endosperm and a low level or activity of total SBEII protein or SBEIIa protein that is 2% to 30% of the level or activity of total SBEII or SBEIIa protein in a wild-type wheat grain, and wherein the grain comprises an amylose content of at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) as a proportion of the total starch in the grain, and ii) extracting the starch from the grain, thereby producing the starch.

The present invention also provides a method of trading wheat grain, comprising obtaining wheat grain of the invention, and trading the obtained wheat grain for pecuniary gain.

In some embodiments, obtaining the wheat gain comprises cultivating or harvesting the wheat grain.

In some embodiments, obtaining the wheat grain comprises harvesting the wheat grain.

In some embodiments, obtaining the wheat grain further comprises storing the wheat grain.

In some embodiments, obtaining the wheat grain further comprises transporting the wheat gain to a different location.

The above summary is not and should not be seen in any way as an exhaustive recitation of all embodiments of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is a representation showing an alignment of SBE IIa protein alignment (AAK26821.1 (SEQ ID N0:3) is from the D genome, CAR95900.1 (SEQ ID N0:2) from the B genome and CAA72154 (SEQ ID N0:1) from the A genome). Dots in the alignment indicate the identical amino acid is present as in the uppermost sequence.

FIG. **2** is a representation showing an alignment of SBEIIb amino acid sequences encoded by exons 1 to 3 from the A (SEQ ID N0:4), B (SEQ ID N0:5) and D genomes (amino acids 1-152 of SEQ ID N0:6) of wheat. Dashes indicate amino acids are present in the protein but the sequence not known, dots in the alignment indicate the identical amino acid is present as in the uppermost sequence.

FIG. **3** is a representation of an alignment of SBEII amino acid sequences. SBE IIb turgidum A/B genome (SEQ ID N0:150).

FIG. **4** is a graphical representation showing a scatter plot of amylase content of transgenic mutant lines (see Example 5).

FIG. **5** is a graphical representation of data showing an amylose model derived from behaviour of SBEII transgenic lines.

FIG. **6** is a graphical representation of data showing an amylose model derived from behaviour of SBEII transgenic line.

FIG. **7** is a representation showing an alignment of DNA sequences of the exons 12 to 14 region of homoeologous SBEIIa genes obtained from the wheat variety Chara. The nucleotide sequence for the Chara B genome fragment (SEQ ID NO:151) is shown in its entirety, while the corresponding nucleotides for the homoeologous A and D genome fragments are shown only where there are polymorphisms. Dots indicate the corresponding nucleotides are identical to the Chara B genome fragment. Dashes indicate that the corresponding nucleotide is absent from the sequence.

FIG. **8** is a representation showing an alignment of DNA sequences of the intron 3 region of SBEIIa genes obtained from the wheat varieties Sunco and Tasman. The nucleotide sequence for the Tasman D genome fragment (SEQ ID NO:152) is shown in its entirety, while the corresponding nucleotides for the homoeologous fragments are shown only where there are polymorphisms. Dots indicate the corresponding nucleotides are identical to the Tasman D genome fragment. Dashes indicate that the corresponding nucleotide is absent from the sequence.

FIG. **9** is a representation showing an alignment of DNA sequences of the exon 3 region of homoeologous SBEIIa genes obtained from the wheat variety Chinese Spring. The nucleotide sequence for the Chinese Spring D genome fragment (SEQ ID NO:153) is shown in its entirety, while the corresponding nucleotides for the homoeologous A and 8 genome fragments are shown only where there are polymorphisms. Dots indicate the corresponding nucleotides are identical to the Chinese Spring D genome fragment.

FIG. **10** is a representation showing a DNA sequence of exon 1 region of SBEIIa gene from the hexaploid wheat variety Chinese Spring (SEQ ID NO:154).

FIG. **11** is a representation showing a PCR amplification of the region spanning exons 12-14 of SBEIIa genes from CS nullisomic-tetrasomic lines. The line designated BDD is null for A genome, ADD is a null for B genome and AAB is a null for D genome.

FIG. **12** is photographic representation of a Western blot showing SBEIIa protein expression in developing endosperms from the line S28. Protein extracts from endosperms were assayed by Western blot analysis as described in Example 1, using SBEIIa-specific antibodies. The last lane on the right-hand side shows the bands appearing from wild-type endosperm (variety NB1). The positions of SBEIIa proteins encoded by the A, B and D genomes are indicated.

FIG. **13** is a plot of mobility ratio of interacting SBEIIa in the absence (m0) and presence (m) of β-limit dextrin in 1-D Native PAGE against the concentration of β-limit dextrin (S). The dissociation constant (Kd) is derived from the equation m0/m=1+[S]/Kd.

FIG. **14** shows the relationship of amylose content and enzyme resistant starch in pooled wheat starch samples derived from transgenic wheat lines described in Example 2

FIG. **15** provides scatter plot representations of NIRS-predicted and biochemical reference values for apparent amylose content in wheat single seeds.

FIG. **16** is a graphical representation showing apparent amylose content distribution on WM and WMC populations as determined by NIRS.

FIGS. **17**(*a*) and (*b*) are graphical representations of data showing the effect of adding increasing quantities of wheat lines on water absorption (a) and Mixograph mixing times (b).

FIGS. **18**(*a*) and (*b*) are graphical representations of data illustrating the effect of adding increasing quantities of high amylose wheat flour on Resistant Starch (a) and predicted GI (b) (HI %) of small scale bread loaves.

BRIEF DESCRIPTION OF THE TABLES

Table 1 provides starch branching enzyme genes characterized from cereals.

Table 2 provides an amino acid sub-classification.

Table 3 provides exemplary amino acid substitutions.

Table 4 provides genome specific primers for wheat SBEIIa gene.

Table 5 provides nucleotide sequences of genome specific primers for SBEIIa.

Table 6 provides primers designed to amplify parts of the SBEIIa gene specifically from the A genome of wheat.

Table 7 provides primers designed to amplify parts of the SBEIIa gene specifically from the B genome of wheat.

Table 8 provides primers designed to amplify parts of the SBEIIa gene specifically from the D genome of wheat.

Table 9 provides genome specific primers for wheat SBEIIb gene.

Table 10 provides nucleotide sequences of genome specific primers for SBEIIb.

Table 11 provides total SBEII and SBEIIa and SBEIIb expression and amylose content of RNAi lines of wheat as described in Example 4.

Table 12 provides a list of microsatellite markers tested in the mutants as described in Example 5.

Table 13 provides mutants identified from HIB population and microsatellite mapping data as described in Example 5.

Table 14 provides a description of double null mutants of SBEII identified as described in Example 5.

Table 15 provides a description of crosses performed between double and single null mutants as described in Example 5.

Table 16 provides tabulation of amylose content in grain starch of triple nulls mutants as described in Example 5.

Table 17 provides fertility observations on F2 progeny plants.

Table 18 provides SBEII allelic composition and amylose proportion data for double nulls identified.

Table 19 provides details of further crosses between single and double null mutants.

Table 20 provides observed frequency of genotypes of normally germinating grain from an A2B2D2 cross. Numbers in parentheses indicate the expected frequency based on Mendelian segregation.

Table 21 provides further crosses between single and double null mutants.

Table 22 provides putative double and triple null mutants in SBEIIa genes identified in an initial screen using dominant markers.

Table 23 provides starch characterisation of grain starch from transgenic wheat lines.

Table 24 provides molecular weight distribution of starch fractions from wheat transgenic lines.

Table 25 provides RVA parameters of hp5'-SBEIIa transgenic wheat starch.

Table 26 provides DSC parameters of gelatinisation peak of hp5'-SBEIIa transgenic wheat starch compared to the control NB 1.

Table 27 provides RS content in rolled and flaked grain products.

Table 28 provides resistant starch content in food products at varying level of incorporation of high amylose wheat (HAW).

Table 29 provides genome-specific primers referred to in Example 18.

DETAILED DESCRIPTION

The present invention is based in part on the observations made in the experiments described herein that wheat plants completely lacking SBEIIa activity throughout the plant could not be recovered in crosses designed to produce them, indeed the complete lack of SBEIIa was concluded to be lethal to seed development and/or fertility. This was surprising since previous studies have shown that single null mutants

in SBEIIa could readily be obtained in wheat and were fertile. Moreover, it was observed that the minimum level of SBEIIa activity that needed to be retained in the wheat plant to produce normal, viable seed was about 2% of the wild-type level.

It was also observed that mutant plants and grain comprising at least one point mutation in an SBEIIa gene were favoured over plants and grain which had deletions in each of the SBEIIa genes for combining mutant SBEIIa genes, in particular to obtain phenotypically normal, male and female fertile plants and grain which germinated at rates similar to wild-type grain. One possible explanation of this observation was that deletions tend to remove important genetic elements adjacent to the SBEIIa genes.

It was also observed that to obtain an amylose content of at least 50% (w/w) in the grain starch, at which level the amount of resistant starch and associated health benefits were increased substantially, the total SBEII activity and particularly the SBEIIa activity in the grain needed to be reduced to below 30% of the wild-type level.

Furthermore, it was determined that, in hexaploid wheat, reducing the level and/or activity of SBEII protein from each of three homoeologous SBEIIa genes or from at least two homoeologous SBEIIa genes and two or three homoeologous SBEIIb genes leads to a substantial non-linear increase in the proportion of amylose in starch of the wheat endosperm compared to plants having null mutation in two homoeologous SBEIIa genes. This non-linear relationship between amylose content and SBEII levels in grain of hexaploid wheat is illustrated graphically in FIGS. **5** and **6**.

By studying partial and complete loss of function mutations in combinations of SBEIIa and/or SBEIIb alleles from A, B and D genomes, the role of multiple SBEII genes in modulating starch characteristics has been established. Specifically, the number of mutant alleles and combinations of mutant alleles required to obtain fertile wheat plants having very high levels of amylose has been investigated and determined.

The synthesis of starch in the endosperm of higher plants including wheat is carried out by a suite of enzymes that catalyse four key steps. Firstly, ADP-glucose pyrophosphorylase (EC 2.7.7.27) activates the monomer precursor of starch through the synthesis of ADP-glucose from G-1-P and ATP. Secondly, the activated glucosyl donor, ADP-glucose, is transferred to the non-reducing end of a pre-existing α(1-4) linkage by starch synthases (EC 2.4.1.24). Thirdly, starch branching enzymes introduce branch points through the cleavage of a region of α(1-4) linked glucan followed by transfer of the cleaved chain to an acceptor chain, forming a new α(1-6) linkage. Starch branching enzymes are the only enzymes that can introduce the α(1-6) linkages into α-polyglucans and therefore play an essential role in the formation of amylopectin. Fourthly, starch debranching enzymes (EC 2.4.4.18) remove some of the branch linkages.

Starch is the major storage carbohydrate in plants such as cereals, including wheat. Starch is synthesized in the amyloplasts and formed and stored in granules in the developing storage organ such as grain; it is referred to herein as "storage starch" or "grain starch". In cereal grains, the vast majority of the storage starch is deposited in the endosperm. "Starch" is defined herein as polysaccharide composed of glucopyranose units polymerized through a combination of both α(1-4) and α(1-6) linkages. The polydisperse molecules of starch are classified as belonging to two component fractions, known as amylose and amylopectin, on the basis of their degree of polymerization (DP) and the ratio of α(1-6) to α(1-4) linkages. Grain starch from wild-type cereal plants, including from wheat, comprises about 20%-30% of amylose and about 70%-80% of amylopectin.

"Amylose" is defined herein as including essentially linear molecules of α(1,4) linked glucosidic (glucopyranose) units, sometimes referred to as "true amylose", and amylose-like long-chain starch which is sometimes referred to as "intermediate material" or "amylose-like amylopectin" which appears as iodine-binding material in an iodometric assay along with true amylose (Takeda et al., 1993b; Fergason, 1994). Typically, the linear molecules in true amylose have a DP of between 500 and 5000 and contain less than 1% α(1-6) linkages. Recent studies have shown that about 0.1% of α(1-6)-glycosidic branching sites may occur in amylose, therefore it is described as "essentially linear". In contrast, amylopectin is a much larger molecule with a DP ranging from 5000 to 50,000 and contains 4-5% α(1-6) linkages. Amylopectin molecules are therefore more highly branched. Amylose has a helical conformation with a molecular weight of about 10^4 to about 10^6 Daltons while amylopectin has a molecular weight of about 10^7 to about 10^8 Daltons. These two types of starch can readily be distinguished or separated by methods well known in the art.

The proportion of amylose in the starch as defined herein is on a weight/weight (w/w) basis, i.e. the weight of amylose as a percentage of the weight of total starch extractable from the grain, with respect to the starch prior to any fractionation into amylose and amylopectin fractions. The terms "proportion of amylose in the starch" and "amylose content" when used herein in the context of the grain, flour or other product of the invention are essentially interchangeable terms. Amylose content may be determined by any of the methods known in the art including size exclusion high-performance liquid chromatography (HPLC), for example in 90% (w/v) DMSO, concanavalin A methods (Megazyme Int, Ireland), or preferably by an iodometric method, for example as described in Example 1. The HPLC method may involve debranching of the starch (Batey and Curtin, 1996) or not involve debranching. It will be appreciated that methods such as the HPLC method of Batey and Curtin, 1996 which assay only the "true amylose" may underestimate the amylose content as defined herein. Methods such as HPLC or gel permeation chromatography depend on fractionation of the starch into the amylose and amylopectin fractions, while iodometric methods depend on differential iodine binding and therefore do not require fractionation.

From the grain weight and amylose content, the amount of amylose deposited per grain can be calculated and compared for test and control lines.

Starch is initially synthesized and accumulated in the leaves and other green tissues of a plant as a product of photosynthesis. This starch is referred to herein as "transitory starch" or the like because, in contrast to seed or tuber starch, it accumulates in the plastids of the photosynthetic tissues during the day and is degraded at least during the night. At night, transitory starch is hydrolysed to sugars which are transported, primarily as sucrose, from the source tissues to sink tissues for use in growth of the plant, as an energy source for metabolism or for storage in tissues as storage starch.

As used herein, "starch synthase" means an enzyme that transfers ADP-glucose to the non-reducing end of a pre-existing α1-4 linkages. Four classes of starch synthase are found in the cereal endosperm, an isoform exclusively localised within the starch granule, granule-bound starch synthase (GBSS), two forms that are partitioned between the granule and the soluble fraction (SSI, Li et al., 1999a; SSII, Li et al., 1999b) and a fourth form that is entirely located in the

soluble fraction, SSIII (Cao et al., 2000; Li et al., 1999b; Li et al., 2000). GBSS has been shown to be essential for amylose synthesis (Shure et al., 1983), and mutations in SSII and SSIII have been shown to alter amylopectin structure (Gao et al., 1998; Craig et al., 1998). Mutants in cereals which lack GBSS also lack true amylose and so accumulate only amylopectin; these are commonly referred to as "waxy" mutants. No mutations defining a role for SSI activity have been described. Amyloepectin synthesis is more complex than amylose synthesis, requiring a combination of starch synthases other than GBSS, multiple starch branching enzymes and debranching enzyme.

As used herein, "debranching enzyme" means an enzyme that removes some of the branches of amylopectin formed by starch branching enzymes. Two types of debranching enzymes are present in higher plants and are defined on the basis of their substrate specificities, isoamylase type debranching enzymes, and pullulanase type debranching enzymes (Myers et al., 2000). Sugary-1 mutations in maize and rice are associated with deficiency of both debranching enzymes (James et al., 1995; Kubo et al., 1999) however the causal mutation maps to the same location as the isoamylase-type debranching enzyme gene.

Examples of genes encoding starch branching enzymes from cereals including wheat are given in Table 1. As used herein, "starch branching enzyme" means an enzyme that introduces α-1,6 glycosidic bonds between chains of glucose residues (EC 2.4.1.18). Three forms of starch branching enzyme are expressed in cereals such as rice, maize, barley and wheat, including in the developing cereal endosperm, namely starch branching enzyme I (SBEI), starch branching enzyme IIa (SBEIIa) and starch branching enzyme IIb (SBEIIb) (Hedman and Boyer, 1982; Boyer and Preiss, 1978; Mizuno et al., 1992, Sun et al., 1997). Genomic and cDNA sequences for genes encoding these enzymes have been characterized for rice, barley and wheat (Table 1). Sequence alignment reveals a high degree of sequence similarity at both the nucleotide and amino acid levels, but also the sequence differences and allows the grouping into the SBEI, SBEIIa and SBEIIb classes. SBEIIa and SBEIIb from any one species generally exhibit around 80% amino acid sequence identity to each other, particularly in the central regions of the genes. SBEIIa and SBEIIb may also be distinguished by their expression patterns, but this differs in different species. In maize, SBEIIb is most highly expressed in endosperm while SBEIIa is present in every tissue of the plant. In barley, both SBEIIa and SBEIIb are present in about equal amounts in the endosperm, while in wheat endoperm, SBEIIa is expressed about 4-fold more highly than SBEIIb. Therefore, the cereal species show significant differences in SBEIIa and SBEIIb expression, and conclusions drawn in one species cannot readily be applied to another species. In wheat, SBEIIa and SBEIIb proteins are different in size (see below) and this is a convenient way to distinguish them. Specific antibodies may also be used to distinguish them.

In maize, high amylose phenotypes have been shown to result from lesions in the SBEIIb gene, also known as the amylase extender (ae) gene (Boyer and Preiss, 1981, Mizuno et al., 1993; Nishi et al, 2001). In these SBEIIb mutants, endosperm starch grains showed an abnormal morphology, amylose content was significantly elevated, the branch frequency of the residual amylopectin was reduced and the proportion of short chains (<DP17, especially DP8-12) was lower. Moreover, the gelatinisation temperature of the starch was increased. In addition, there was a significant pool of material that was defined as "intermediate" between amylose and amylopectin (Boyer et al., 1980; Takeda, et al., 1993b). In contrast, maize plants mutant in the SBEIIa gene due to a mutator (Mu) insertional element and consequently lacking in SBEIIa protein expression were indistinguishable from wild-type plants in the branching of endosperm starch (Blauth et al., 2001), although they were altered in leaf starch. Similarly, rice plants deficient in SBEIIa activity exhibited no significant change in the amylopectin chain profile in endosperm (Nakamura, 2002), while mutants in SBEIIb showed a modest increase in amylose levels, up to about 35% in indica backgrounds and up to 25-30% in a japonica background (Mizuno et al., 1993; Nishi et al., 2001). In both maize and rice, the SBEIIa and SBEIIb genes are not linked in the genome. In barley, a gene silencing construct which reduced both SBEIIa and SBEIIb expression in endosperm was used to generate high amylose barley grain (Regina et al., 2010).

In developing wheat endosperm, SBEI (Morell et al., 1997) is found exclusively in the soluble fraction (amyloplast stroma), while SBEIIa and SBEIIb are found in both soluble and starch-granule associated fractions in endosperm (Rahman et al., 1995). In wheat, apparent gene duplication events have increased the number of SBEI genes in each genome (Rahman et al., 1999). The elimination of greater than 97% of the SBEI activity by combining mutations in the highest expressing forms of the SBEI genes from the A, B and D genomes had no measurable impact on starch structure or functionality (Regina et al., 2004). In contrast, reduction of SBEIIa expression by a gene silencing construct in wheat resulted in high amylose levels (>70%), while a corresponding construct that reduced SBEIIb expression but not SBEIIa had minimal effect (Regina et al., 2006).

Starch branching enzyme (SBE) activity may be measured by enzyme assay, for example by the phosphorylase stimulation assay (Boyer and Preiss, 1978). This assay measures the stimulation by SBE of the incorporation of glucose 1-phosphate into methanol-insoluble polymer (α-D-glucan) by phosphorylase A. SBE activity can be measured by the iodine stain assay, which measures the decrease in the absorbency of a glucan-polyiodine complex resulting from branching of glucan polymers. SBE activity can also be assayed by the branch linkage assay which measures the generation of reducing ends from reduced amylose as substrate, following isoamylose digestion (Takeda et al., 1993a). Preferably, the activity is measured in the absence of SBEI activity. Isoforms of SBE show different substrate specificities, for example SBEI exhibits higher activity in branching amylose, while SBEIIa and SBEIIb show higher rates of branching with an amylopectin substrate. The isoforms may also be distinguished on the basis of the length of the glucan chain that is transferred. SBE protein may also be measured by using specific antibodies such as those described herein. The SBEII activity may be measured during grain development in the developing endosperm. Alternatively, SBEII levels are measured in the mature grain where the protein is still present and can be assayed by immunological methods.

In some embodiments, the level or activity of SBEII or SBEIIa may be assessed by assessing transcript levels such as by Northern or RT-PCR analysis. In a preferred method, the amount of SBEIIa protein in grain or developing endosperm is measured by separating the proteins in extracts of the grain/endosperm on gels by electrophoresis, then transferring the proteins to a membrane by Western blotting, followed by quantitative detection of the protein on the membrane using specific antibodies ("Western blot analysis"). This is exemplified in Example 11.

As shown herein, developing hexaploid wheat endosperm expresses SBEIIa and SBEIIb from each of the A, B and D genomes. Tetraploid wheat expresses SBEIIa and SBEIIb

from each of the A and B genomes. As used herein, "SBEIIa expressed from the A genome" or "SBEIIa-A" means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 1 or which is at least 99% identical to the amino acid sequence set forth in SEQ ID NO: 1 or comprising such a sequence. The amino acid sequence of SEQ ID NO: 1 (Genbank Accession No. CAA72154) corresponds to an SBEIIa expressed from the A genome of wheat, which is used herein as the reference sequence for wild-type SBEIIa-A. The protein of SEQ ID NO: 1 is 823 amino acids long. Active variants of this enzyme exist in wheat, for example in cultivar Cheyenne, see Accession No. AF286319 which is 99.88% (822/823) identical to SEQ ID NO. 1. Such variants are included in "SBEIIa-A" provided they have essentially wild-type starch branching enzyme activity as for SEQ ID NO: 1.

As used herein, "SBEIIa expressed from the B genome" or "SBEIIa-B" means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 2 or which is at least 99% identical to the amino acid sequence set forth in SEQ ID NO: 2 or comprising such a sequence. The amino acid sequence of SEQ ID NO: 2 (Genbank Accession No. CAR95900) corresponds to the SBEIIa expressed from the B genome of wheat variety Chinese Spring, which is used herein as the reference sequence for wild-type SBEIIa-B. The protein of SEQ ID NO: 2 is 823 amino acids long. Active variants of this enzyme may exist in wheat and are included in SBEIIa-B provided they have essentially wild-type starch branching enzyme activity as for SEQ ID NO: 2. SEQ ID NO: 2 is 98.42% (811/824) identical to SEQ ID NO: 1. The alignment of the amino acid sequences in FIG. **1** shows the amino acid differences which may be used to distinguish the proteins or to classify variants as SBEIIa-A or SBEIIa-B.

As used herein, "SBEIIa expressed from the D genome" or "SBEIIa-D" means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 3 or which is at least 98% identical to the amino acid sequence set forth in SEQ ID NO: 3 or comprising such a sequence. The amino acid sequence of SEQ ID NO: 3 (Genbank Accession No. AAK26821) corresponds to the SBEIIa expressed from the D genome in *A. tauschii*, a likely progenitor of the D genome of hexaploid wheat, which is used herein as the reference sequence for wild-type SBEIIa-D. The protein of SEQ ID NO: 3 is 819 amino acids long. Active variants of this enzyme may exist in wheat and are included in SBEIIa-D provided they have essentially wild-type starch branching enzyme activity as for SEQ ID NO: 3. SEQ ID NO: 3 is 97.57% (803/823) identical to SEQ ID NO: 1 and 97.81% (805/823) identical to SEQ ID NO: 2. The alignment of the amino acid sequences in FIG. **1** shows amino acid differences which may be used to distinguish the proteins or to classify variants as SBEIIa-A, SBEIIa-B or SBEIIa-D.

When comparing amino acid sequences to determine the percentage identity in this context, for example by Blastn, the full length sequences should be compared, and gaps in a sequence counted as amino acid differences.

As used herein, an "SBEIIa protein" includes protein variants which have reduced or no starch branching enzyme activity, as well as the proteins having essentially wild-type enzyme activity. It is also understood that SBEIIa proteins may be present in grain, particularly dormant grain as commonly harvested commercially, but in an inactive state because of the physiological conditions in the grain. Such proteins are included in "SBEIIa proteins" as used herein. The SBEIIa proteins may be enzymatically active during only part of grain development, in particular in developing endosperm when storage starch is typically deposited, but in inactive state otherwise. Such SBEIIa protein may be detected and quantitated readily using immunological methods such as Western blot analysis. An "SBEIIb protein" as used herein has an analogous meaning.

As used herein, "SBEIIb expressed from the A genome" or "SBEIIb-A" means a starch branching enzyme comprising the amino acid sequence set forth in SEQ ID NO: 4 or which is at least 98% identical to the amino acid sequence set forth in SEQ ID NO: 4 or comprising such a sequence. The amino acid sequence of SEQ ID NO: 4 corresponds to the amino terminal sequence of SBEIIb expressed from the A genome of wheat, which is used herein as the reference sequence for wild-type SBEIIb-A.

As used herein, "SBEIIb expressed from the B genome" or "SBEIIb-B" means a starch branching enzyme comprising the amino acid sequence set forth in SEQ ID NO: 5 or which is at least 98% identical to the amino acid sequence set forth in SEQ ID NO: 5 or comprising such a sequence. The amino acid sequence SEQ ID NO: 5, which is used herein as the reference sequence for wild-type SBEIIb-B, is a partial amino acid sequence encoded by exons 2-3 of the SBEIIb-B gene in wheat. A variant SBEIIb-B sequence is the amino acid sequence encoded by the nucleotide sequence of Accession No. AK335378 isolated from cv. Chinese Spring.

As used herein, "SBEIIb expressed from the D genome" or "SBEIIb-D" means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 6 or which is at least 98% identical to the amino acid sequence set forth in SEQ ID NO: 6 or comprising such a sequence. The amino acid sequence of SEQ ID NO: 6 (Genbank Accession No. AAW80631) corresponds to the SBEIIb expressed from the D genome of *A. tauschii*, a likely progenitor of the D genome of hexaploid wheat, and is used herein as the reference sequence for wild-type SBEIIa-D. Active variants of this enzyme exist in wheat and are included in SBEIIb-D provided they have essentially wild-type starch branching enzyme activity as for SEQ ID NO: 6. For example, SEQ ID NO: 4 of US patent application publication No, 20050074891, beginning at the first methionine, shows the amino acid sequence of a SBEIIb-D protein which is 99.5% identical to SEQ ID NO: 6 in this application. The alignment of the amino acid sequences in FIG. **2** shows amino acid differences which may be used to distinguish SBEIIb proteins or to classify variants as SBEIIb-A, SBEIIb-B or SBEIIb-D.

Thus, "wild-type" as used herein when referring to SBEIIa-A means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 1; "wild-type" as used herein when referring to SBEIIa-B means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 2; "wild-type" as used herein when referring to SBEIIa-D means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 3; "wild-type" as used herein when referring to SBEIIb-A means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 4; "wild-type" as used herein when referring to SBEIIb-13 means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 5; and, "wild-type" as used herein when referring to SBEIIb-D means a starch branching enzyme whose amino acid sequence is set forth in SEQ ID NO: 6.

As used herein, the terms "wheat SBEIIa gene" and "wheat SBEIIb gene" refer to the genes that encode functional SBEIIa or SBEIIb enzymes, respectively, in wheat, including homologous genes present in other wheat varieties, and also mutant forms of the genes which encode enzymes with reduced activity or undetectable activity. These include, but are not limited to, the wheat SBEII genes which have been cloned, including the genomic and cDNA sequences listed in

Table 1. The genes as used herein encompasses mutant forms which do not encode any proteins at all, in which case the mutant forms represent null alleles of the genes.

An "endogenous SHEII gene" refers to an SBEII gene which is in its native location in the wheat genome, including wild-type and mutant forms. In contrast, the terms "isolated SBEII gene" and "exogenous SBEII gene" refer to an SBEII gene which is not in its native location, for example having been cloned, synthesized, comprised in a vector or in the form of a transgene in a cell, preferably as transgene in a transgenic wheat plant. The SBEII gene in this context may be any of the specific forms as described as follows.

As used herein, "the SBEIIa gene on the A genome of wheat" or "SBEIIa-A gene" means any polynucleotide which encodes SBEIIa-A as defined herein or which is derived from a polynucleotide which encodes SBEIIa-A, including naturally occurring polynucleotides, sequence variants or synthetic polynucleotides, including "wild-type SBEIIa-A gene(s)" which encode an SBEIIa-A with essentially wild-type activity, and "mutant SBEIIa-A gene(s)" which do not encode an SBEIIa-A with essentially wild-type activity but are recognizably derived from a wild-type SBEIIa-A gene. Comparison of the nucleotide sequence of a mutant form of an SBEII gene with a suite of wild-type SBEII genes is used to determine which of the SBEII genes it is derived from and so to classify it. For example, a mutant SBEII gene is considered to be a mutant SBEIIa-A gene if its nucleotide sequence is more closely related, i.e. having a higher degree of sequence identity, to a wild-type SBEIIa-A gene than to any other SBEII gene. A mutant SBEIIa-A gene encodes a SBE with reduced starch branching enzyme activity (partial mutant), or a protein which lacks SBE activity or no protein at all (null mutant gene). An exemplary nucleotide sequence of a cDNA corresponding to a SBEIIa-A gene is given in Genbank Accession No. Y11282. Sequences of parts of SBEIIa-A genes are also given herein as referred to in FIGS. **7**, **8**, **9** and **10** and SEQ ID NOs 13, 14 and 15.

As used herein, the terms "SBEIIa expressed from the B genome" or "SBEIIa-B", "SBEIIa expressed from the D genome" or "SBEIIa-D", "SBEIIb expressed from the A genome" or "SBEIIb-A", "SBEIIb expressed from the B genome" or "SBEIIb-B" and "SBEIIb expressed from the D genome" or "SBEIIb-D" have corresponding meanings to that for SBEIIa-A in the previous paragraph.

Illustrative partial SBEIIb-A, SBEIIb-B and SBEIIb-D protein sequences are provided in FIG. **2**. Illustrative SBEIIb-A amino acid sequences are set out in SEQ ID NO: 1 and SEQ ID NO: 4 (amino terminal sequence encoded by exon 1-3). Illustrative SBEIIb-B amino acid sequences are set out in SEQ ID NO: 2 and SEQ ID NO: 5. Illustrative SBEIIb-D amino acid sequences are set out in SEQ ID NO: 3 and SEQ ID NO: 6 and SEQ ID NO: 9.

The SBEII genes as defined above include any regulatory sequences that are 5' or 3' of the transcribed region, including the promoter region, that regulate the expression of the associated transcribed region, and introns within the transcribed regions.

It would be understood that there is natural variation in the sequences of SBEIIa and SBEIIb genes from different wheat varieties. The homologous genes are readily recognizable by the skilled artisan on the basis of sequence identity. The degree of sequence identity between homologous SBEIIa genes or the proteins is thought to be at least 90%, similarly for SBEIIb genes or proteins. Wheat SBEIIa genes are about 80% identical in sequence to wheat SBEIIb genes. The encoded proteins are also about 80% identical in sequence.

An allele is a variant of a gene at a single genetic locus. A diploid organism has two sets of chromosomes. Each chromosome has one copy of each gene (one allele). If both alleles are the same the organism is homozygous with respect to that gene, if the alleles are different, the organism is heterozygous with respect to that gene. The interaction between alleles at a locus is generally described as dominant or recessive. A loss of function mutation is a mutation in an allele leading to no or a reduced detectable level or activity of SBEII, SBEIIa or SBEIIb enzyme in the grain. The mutation may mean, for example, that no or less RNA is transcribed from the gene comprising the mutation or that the protein produced has no or reduced activity. Alleles that do not encode or are not capable of leading to the production any active enzyme are null alleles. A loss of function mutation, which includes a partial loss of function mutation in an allele, means a mutation in the allele leading to a reduced level or activity of SBEII, SBEIIa or SBEIIb enzyme in the grain. The mutation in the allele may mean, for example, that less protein having wild-type or reduced activity is translated or that wild-type or reduced levels of transcription are followed by translation of an enzyme with reduced enzyme activity. A "reduced" amount or level of protein means reduced relative to the amount or level produced by the corresponding wild-type allele. A "reduced" activity means reduced relative to the corresponding wild-type SBEII, SBEIIa or SBEIIb enzyme. Different alleles in the embryo may have the same or a different mutation and different alleles may be combined using methods known in the art. In some embodiments, the amount of SBEIIa protein or SBEIIb protein is reduced because there is less transcription or translation of the SBEIIa gene or SBEIIb gene, respectively. In some embodiments, the amount by weight of SBEIIa protein or SBEIIb protein is reduced even though there is a wild-type number of SBEIIa protein molecules or SBEIIb protein molecules in the grain, because some of the proteins produced are shorter than wild-type SBEIIa protein or SBEIIb protein, e.g. the mutant SBEIIa protein or SBEIIb protein is truncated due to a premature translation termination signal.

Representative starch biosynthesis genes that have been cloned from cereals are listed in Table 1.

As used herein, "two identical alleles of an SBEIIa-A gene", means that the two alleles of the SBEIIa-A gene are identical to each other; "two identical alleles of an SBEIIa-B gene", means that the two alleles of the SBEIIa-B gene are identical to each other, "two identical alleles of an SBEIIa-D gene", means that the two alleles of the SBEIIa-D gene are identical to each other; "two identical alleles of an SBEIIb-A gene", means that the two alleles of the SBEIIb-A gene are identical to each other; "two identical alleles of an SBEIIb-B gene", means that the two alleles of the SBEIIb-B gene are identical to each other; and, "two identical alleles of an SBEIIb-D gene", means that the two alleles of the SBEIIb-D gene are identical to each other.

The wheat plants of the invention can be produced and identified after mutagenesis. This may provide a wheat plant which is non-transgenic, which is desirable in some markets, or which is free of any exogenous nucleic acid molecule which reduces expression of an SBEIIa gene. Mutant wheat plants having a mutation in a single SBEII gene which can be combined by crossing and selection with other SBEII mutations to generate the wheat plants of the invention can be either synthetic, for example, by performing site-directed mutagenesis on the nucleic acid, or induced by mutagenic treatment, or may be naturally occurring, i.e. isolated from a natural source. Generally, a progenitor plant cell, tissue, seed or plant may be subjected to mutagenesis to produce single or

multiple mutations, such as nucleotide substitutions, deletions, additions and/or codon modification. Preferred wheat plants and grain of the invention comprise at least one introduced SBEII mutation, more preferably two or more introduced SBEII mutations, and may comprise no mutations from a natural source i.e. all of the mutant SBEIIa and SBEIIb alleles in the plant were obtained by synthetic means or by mutagenic treatment.

Mutagenesis can be achieved by chemical or radiation means, for example EMS or sodium azide (Zwar and Chandler, 1995) treatment of seed, or gamma irradiation, well know in the art. Chemical mutagenesis tends to favour nucleotide substitutions rather than deletions. Heavy ion beam (HIB) irradiation is known as an effective technique for mutation breeding to produce new plant cultivars, see for example Hayashi et al., 2007 and Kazama et al, 2008. Ion beam irradiation has two physical factors, the dose (gy) and LET (linear energy transfer, keV/um) for biological effects that determine the amount of DNA damage and the size of DNA deletion, and these can be adjusted according to the desired extent of mutagenesis. HIB generates a collection of mutants, many of them comprising deletions that may be screened for mutations in specific SBEII genes as shown in the Examples. Mutants which are identified may be backcrossed with non-mutated wheat plants as recurrent parents in order to remove and therefore reduce the effect of unlinked mutations in the mutagenised genome, see Example 9.

Biological agents useful in producing site-specific mutants include enzymes that include double stranded breaks in DNA that stimulate endogenous repair mechanisms. These include endonucleases, zinc finger nucleases, transposases and site-specific recombinases. Zinc finger nucleases (ZFNs), for example, facilitate site-specific cleavage within a genome allowing endogenous or other end-joining repair mechanisms to introduce deletions or insertions to repair the gap. Zinc finger nuclease technology is reviewed in Le Provost a al, 2009, See also Durai a al., 2005 and Liu a al., 2010.

Isolation of mutants may be achieved by screening mutagenised plants or seed. For example, a mutagenized population of wheat may be screened directly for the SBEIIa and/or SBEIIb genotype or indirectly by screening for a phenotype that results from mutations in the SBEII genes. Screening directly for the genotype preferably includes assaying for the presence of mutations in the SBEII genes, which may be observed in PCR assays by the absence of specific SBEIIa or SBEIIb markers as expected when some of the genes are deleted, or heteroduplex based assays as in Tilling. Screening for the phenotype may comprise screening for a loss or reduction in amount of one or more SBEIIa or SBEIIb proteins by ELISA or affinity chromatography, or increased amylose content in the grain starch. In hexaploid wheat, screening is preferably done in a genotype that already lacks one or two of the SBEII activities, for example in a wheat plant already mutant in the SBEIIa or SBEIIb genes on two of the three genomes, so that a mutant further lacking the functional activity is sought. In tetraploid wheat, screening is preferably done in a genotype that already lacks one SBEII activity, on either the A or B genome, and identifying a mutant which is reduced in the SBEII from the second genome. Affinity chromatography may be carried out as demonstrated in Example 11. Large populations of mutagenised seeds (thousands or tens of thousands of seeds) may be screened for high amylose phenotypes using near infra-red spectroscopy (NIR) as demonstrated in Example 10. Using NIR, a sub-population enriched for high amylose candidates was obtainable. By these means, high throughput screening is readily achievable and allows the isolation of mutants at a frequency of approximately one per several hundred seeds.

Plants and seeds of the invention can be produced using the process known as TILLING (Targeting Induced Local Lesions IN Genomes), in that one or more of the mutations in the wheat plants or grain may be produced by this method. In a first step, introduced mutations such as novel single base pair changes are induced in a population of plants by treating seeds or pollen with a chemical or radiation mutagen, and then advancing plants to a generation where mutations will be stably inherited, typically an M2 generation where homozygotes may be identified. DNA is extracted, and seeds are stored from all members of the population to create a resource that can be accessed repeatedly over time. For a TILLING assay, PCR primers are designed to specifically amplify a single gene target of interest. Next, dye-labeled primers can be used to amplify PCR products from pooled DNA of multiple individuals. These PCR products are denatured and reannealed to allow the formation of mismatched base pairs. Mismatches, or heteroduplexes, represent both naturally occurring single nucleotide polymorphisms (SNPs) (i.e., several plants from the population are likely to carry the same polymorphism) and induced SNPs (i.e., only rare individual plants are likely to display the mutation). After heteroduplex formation, the use of an endonuclease, such as Cel I, that recognizes and cleaves mismatched DNA is the key to discovering novel SNPs within a TILLING population.

Using this approach, many thousands of plants can be screened to identify any individual with a single base change as well as small insertions or deletions (1-30 bp) in any gene or specific region of the genome. Genomic fragments being assayed can range in size anywhere from 0.3 to 1.6 kb. At 8-fold pooling and amplifying 1.4 kb fragments with 96 lanes per assay, this combination allows up to a million base pairs of genomic DNA to be screened per single assay, making TILLING a high-throughput technique. TILLING is further described in Slade and Knauf, 2005, and Henikoff et al., 2004.

In addition to allowing efficient detection of mutations, high-throughput TILLING technology is ideal for the detection of natural polymorphisms. Therefore, interrogating an unknown homologous DNA by heteroduplexing to a known sequence reveals the number and position of polymorphic sites. Both nucleotide changes and small insertions and deletions are identified, including at least some repeat number polymorphisms. This has been called Ecotilling (Comai et al., 2004). Plates containing arrayed ecotypic DNA can be screened rather than pools of DNA from mutagenized plants. Because detection is on gels with nearly base pair resolution and background patterns are uniform across lanes, bands that are of identical size can be matched, thus discovering and genotyping mutations in a single step. In this way, sequencing of the mutant gene is simple and efficient.

Identified mutations may then be introduced into desirable genetic backgrounds by crossing the mutant with a plant of the desired genetic background and performing a suitable number of backcrosses to cross out the originally undesired parent background.

In the context of this application, an "induced mutation" or "introduced mutation" is an artificially induced genetic variation which may be the result of chemical, radiation or biologically-based mutagenesis, for example transposon or T-DNA insertion. Preferred mutations are null mutations such as nonsense mutations, frameshift mutations, deletions, insertional mutations or splice-site variants which completely inactivate the gene. Other preferred mutations are partial mutations which retain some SBEII activity, but less than

wild-type levels of the enzyme. Nucleotide insertional derivatives include 5 and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a site in the nucleotide sequence, either at a predetermined site as is possible with zinc finger nucleases (ZFN) or other homologous recombination methods, or by random insertion with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Preferably, a mutant gene has only a single insertion or deletion of a sequence of nucleotides relative to the wild-type gene. The deletion may be extensive enough to include one or more exons or introns, both exons and introns, an intron-exon boundary, a part of the promoter, the translational start site, or even the entire gene. Deletions may extend far enough to include at least part of, or the whole of, both the SBEIIa and SBEIIb genes on the A, B or D genome, based on the close genetic linkage of the two genes. Insertions or deletions within the exons of the protein coding region of a gene which insert or delete a number of nucleotides which is not an exact multiple of three, thereby causing a change in the reading frame during translation, almost always abolish activity of the mutant gene comprising such insertion or deletion.

Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. The preferred number of nucleotides affected by substitutions in a mutant gene relative to the wild-type gene is a maximum of ten nucleotides, more preferably a maximum of 9, 8, 7, 6, 5, 4, 3, or 2, or most preferably only one nucleotide. Substitutions may be "silent" in that the substitution does not change the amino acid defined by the codon. Nucleotide substitutions may reduce the translation efficiency and thereby reduce the SBEII expression level, for example by reducing the mRNA stability or, if near an exon-intron splice boundary, alter the splicing efficiency. Silent substitutions that do not alter the translation efficiency of a SBEIIa or SBEIIb gene are not expected to alter the activity of the genes and are therefore regarded herein as non-mutant, i.e. such genes are active variants and not encompassed in "mutant alleles". Alternatively, the nucleotide substitution(s) may change the encoded amino acid sequence and thereby alter the activity of the encoded enzyme, particularly if conserved amino acids are substituted for another amino acid which is quite different i.e. a non-conservative substitution. Typical conservative substitutions are those made in accordance with Table 3.

The term "mutation" as used herein does not include silent nucleotide substitutions which do not affect the activity of the gene, and therefore includes only alterations in the gene sequence which affect the gene activity. The term "polymorphism" refers to any change in the nucleotide sequence including such silent nucleotide substitutions. Screening methods may first involve screening for polymorphisms and secondly for mutations within a group of polymorphic variants.

As is understood in the art, hexaploid wheats such as bread wheat comprise three genomes which are commonly designated the A, B and D genomes, while tetraploid wheats such as durum wheat comprise two genomes commonly designated the A and B genomes. Each genome comprises 7 pairs of chromosomes which may be observed by cytological methods during meiosis and thus identified, as is well known in the art.

The terms "plant(s)" and "wheat plant(s)" as used herein as a noun generally refer to whole plants, but when "plant" or "wheat" is used as an adjective, the terms refer to any substance which is present in, obtained from, derived from, or related to a plant or a wheat plant, such as for example, plant organs (e.g. leaves, stems, roots, flowers), single cells (e.g. pollen), seeds, plant cells including for example tissue cultured cells, products produced from the plant such as "wheat flour", "wheat grain", "wheat starch", "wheat starch granules" and the like. Plantlets and germinated seeds from which roots and shoots have emerged are also included within the meaning of "plant". The term "plant parts" as used herein refers to one or more plant tissues or organs which are obtained from a whole plant, preferably a wheat plant. Plant parts include vegetative structures (for example, leaves, stems), roots, floral organs/structures, seed (including embryo, endosperm, and seed coat), plant tissue (for example, vascular tissue, ground tissue, and the like), cells and progeny of the same. The term "plant cell" as used herein refers to a cell obtained from a plant or in a plant, preferably a wheat plant, and includes protoplasts or other cells derived from plants, gamete-producing cells, and cells which regenerate into whole plants. Plant cells may be cells in culture. By "plant tissue" is meant differentiated tissue in a plant or obtained from a plant ("explant") or undifferentiated tissue derived from immature or mature embryos, seeds, roots, shoots, fruits, pollen, and various forms of aggregations of plant cells in culture, such as calli. Plant tissues in or from seeds such as wheat seeds are seed coat, endosperm, scutellum, aleuron layer and embryo.

Cereals as used herein means plants or grain of the monocotyledonous families Poaceae or Graminae which are cultivated for the edible components of their seeds, and includes wheat, barley, maize, oats, rye, rice, sorghum, triticale, millet, buckwheat. Preferably, the cereal plant or grain is wheat or barley plant or grain, more preferably wheat plant or grain. In a further preferred embodiment, the cereal plant is not rice or maize or both of these.

As used herein, the term "wheat" refers to any species of the Genus *Triticum*, including progenitors thereof, as well as progeny thereof produced by crosses with other species. Wheat includes "hexaploid wheat" which has genome organization of AABBDD, comprised of 42 chromosomes, and "tetraploid wheat" which has genome organization of AABB, comprised of 28 chromosomes. Hexaploid wheat includes *T. aestivum, T. spelta, T. mocha, T. compactum, T. sphaerococcum, T. vavilovii*, and interspecies cross thereof. Tetraploid wheat includes *T. durum* (also referred to as durum wheat or *Triticum turgidum* ssp. *durum*), *T. dicoccoides, T. dicoccum, T. polonicum*, and interspecies cross thereof. In addition, the term "wheat" includes possible progenitors of hexaploid or tetraploid *Triticum* sp. such as *T. uartu, T. monococcum or T. boeoticum* for the A genome, *Aegilops speltoides* for the B genome, and *T. tauschii* (also known as *Aegilops squarrosa* or *Aegilops tauschii*) for the D genome. A wheat cultivar for use in the present invention may belong to, but is not limited to, any of the above-listed species. Also encompassed are plants that are produced by conventional techniques using *Triticum* sp. as a parent in a sexual cross with a non-*Triticum* species, such as rye *Secale cereale*, including but not limited to Triticale. Preferably the wheat plant is suitable for commercial production of grain, such as commercial varieties of hexaploid wheat or durum wheat, having suitable agronomic characteristics which are known to those skilled in the art. More preferably the wheat is *Triticum aestivum* ssp. *aestivum* or *Triticum turgidum* ssp. *durum*, and most preferably the wheat is *Triticum aestivum* ssp. *aestivum*, herein also referred to as "breadwheat".

As used herein, the term "barley" refers to any species of the Genus *Hordeum*, including progenitors thereof, as well as progeny thereof produced by crosses with other species. It is preferred that the plant is of a *Hordeum* species which is commercially cultivated such as, for example, a strain or cultivar or variety of *Hordeum vulgare* or suitable for commercial production of grain.

Aspects of the invention provide methods of planting and harvesting wheat grain of the invention, and methods of producing bins of wheat grain of the invention. For instance, after the ground is prepared by plowing and/or certain other methods, the seeds (kernels) are planted either by sowing them broadcast (i.e., by distributing them on the surface of the ground) or by drilling furrows and planting the seeds in rows. To prevent scattering of the kernels, wheat is often harvested before it is fully ripe. There are several steps in harvesting: cutting, or reaping, the stalks; threshing and winnowing, to separate the kernels from the spikes, glumes, and other chaff; sifting and sorting the grain; loading the grain into trucks; and binding the straw. In some embodiments harvested wheat grain may be stored in dry, well-ventilated buildings that keep out insect pests. In some embodiments, harvested wheat grain may be stored for a short time in bins or granaries. The wheat grain may then by hauled to country elevators, tall structures where the grain is dried and stored until it is sold or shipped to terminal elevators. Therefore, embodiments of the invention provide a process of producing bins of wheat grain comprising: a) reaping wheat stalks comprising wheat grain as defined herein; b) threshing and/or winnowing the stalks to separate the grain from the chaff; and c) sifting and/or sorting the grain separated in step b), and loading the sifted and/or sorted grain into bins, thereby producing bins of wheat grain.

The wheat plants of the invention may have many uses other than uses for food or animal feed, for example uses in research or breeding. In seed propagated crops such as wheat, the plants can be self-crossed to produce a plant which is homozygous for the desired genes, or haploid tissues such as developing germ cells can be induced to double the chromosome complement to produce a homozygous plant. The inbred wheat plant of the invention thereby produces seed containing the combination of mutant SBEII alleles which may be homozygous. These seeds can be grown to produce plants that would have the selected phenotype such as, for example, high amylose content in its starch.

The wheat plants of the invention may be crossed with plants containing a more desirable genetic background, and therefore the invention includes the transfer of the low SBEII trait to other genetic backgrounds. After the initial crossing, a suitable number of backcrosses may be carried out to remove a less desirable background. SBEII allele-specific PCR-based markers such as those described herein may be used to screen for or identify progeny plants or grain with the desired combination of alleles, thereby tracking the presence of the alleles in the breeding program. The desired genetic background may include a suitable combination of genes providing commercial yield and other characteristics such as agronomic performance or abiotic stress resistance. The genetic background might also include other altered starch biosynthesis or modification genes, for example genes from other wheat lines. The genetic background may comprise one or more transgenes such as, for example, a gene that confers tolerance to a herbicide such as glyphosate.

The desired genetic background of the wheat plant will include considerations of agronomic yield and other characteristics. Such characteristics might include whether it is desired to have a winter or spring types, agronomic performance, disease resistance and abiotic stress resistance. For Australian use, one might want to cross the altered starch trait of the wheat plant of the invention into wheat cultivars such as Baxter, Kennedy, Janz, Frame, Rosella, Cadoux, Diamondbird or other commonly grown varieties. Other varieties will be suited for other growing regions. It is preferred that the wheat plant of the invention provide a grain yield of at least 80% relative to the yield of the corresponding wild-type variety in at least some growing regions, more preferably at least 85% or at least 90%, and even more preferably at least 95% relative to a wild-type variety having about the same genetic background, grown under the same conditions. Most preferably, the grain yield of the wheat plant of the invention is at least as great as the yield of the wild-type wheat plant having about the same genetic background, grown under the same conditions. The yield can readily be measured in controlled field trials, or in simulated field trials in the greenhouse, preferably in the field.

Marker assisted selection is a well recognised method of selecting for heterozygous plants obtained when backcrossing with a recurrent parent in a classical breeding program. The population of plants in each backcross generation will be heterozygous for the gene(s) of interest normally present in a 1:1 ratio in a backcross population, and the molecular marker can be used to distinguish the two alleles of the gene. By extracting DNA from, for example, young shoots and testing with a specific marker for the introgressed desirable trait, early selection of plants for further backcrossing is made whilst energy and resources are concentrated on fewer plants.

Procedures such as crossing wheat plants, self-fertilising wheat plants or marker-assisted selection are standard procedures and well known in the art. Transferring alleles from tetraploid wheat such as durum wheat to a hexaploid, or other forms of hybridisation, is more difficult but is also known in the art.

To identify the desired phenotypic characteristic, wheat plants that contain a combination of mutant SBEIIa and SBEIIb alleles or other desired genes are typically compared to control plants. When evaluating a phenotypic characteristic associated with enzyme activity such as amylose content in the grain starch, the plants to be tested and control plants are grown under growth chamber, greenhouse, open top chamber and/or field conditions. Identification of a particular phenotypic trait and comparison to controls is based on routine statistical analysis and scoring. Statistical differences between plants lines can be assessed by comparing—enzyme activity between plant lines within each tissue type expressing the enzyme. Expression and activity are compared to growth, development and yield parameters which include plant part morphology, colour, number, size, dimensions, dry and wet weight, ripening, above- and below-ground biomass ratios, and timing, rates and duration of various stages of growth through senescence, including vegetative growth, fruiting, flowering, and soluble carbohydrate content including sucrose, glucose, fructose and starch levels as well as endogenous starch levels. Preferably, the wheat plants of the invention differ from wild-type plants in one or more of these parameters by less than 50%, more preferably less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2% or less than 1% when grown under the same conditions.

As used herein, the term "linked" refers to a marker locus and a second locus being sufficiently close on a chromosome that they will be inherited together in more than 50% of meioses, e.g., not randomly. This definition includes the situation where the marker locus and second locus form part of the same gene. Furthermore, this definition includes the situation where the marker locus comprises a polymorphism that

is responsible for the trait of interest (in other words the marker locus is directly "linked" to the phenotype). The term "genetically linked" as used herein is narrower, only used in relation to where a marker locus and a second locus being sufficiently close on a chromosome that they will be inherited together in more than 50% of meioses. Thus, the percent of recombination observed between the loci per generation (centimorgans (cM)), will be less than 50. In particular embodiments of the invention, genetically linked loci may be 45, 35, 25, 15, 10, 5, 4, 3, 2, or 1 or less cM apart on a chromosome. Preferably, the markers are less than 5 cM or 2 cM apart and most preferably about 0 cM apart. As described in Example 5 herein, the SBEIIa and SBEIIb genes are genetically linked on the long arm of chromosome 2 of each of the wheat genomes, being about 0.5 cM apart, which corresponds to about 100-200 kb in physical distance.

As used herein, the "other genetic markers" may be any molecules which are linked to a desired trait in the wheat plants of the invention. Such markers are well known to those skilled in the art and include molecular markers linked to genes determining traits such disease resistance, yield, plant morphology, grain quality, other dormancy traits such as grain colour, gibberellic acid content in the seed, plant height, flour colour and the like. Examples of such genes are stem-rust resistance genes Sr2 or Sr38, the stripe rust resistance genes Yr10 or Yr17, the nematode resistance genes such as Cre1 and Cre3, alleles at glutenin loci that determine dough strength such as Ax, Bx, Dx, Ay, By and Dy alleles, the Rht genes that determine a semi-dwarf growth habit and therefore lodging resistance (Eagles et al., 2001; Langridge et al., 2001; Sharp et al., 2001).

The wheat plants, wheat plant parts and products therefrom of the invention are preferably non-transgenic for genes that inhibit expression of SBEIIa i.e. they do not comprise a transgene encoding an RNA molecule that reduces expression of the endogenous SBEIIa genes, although in this embodiment they may comprise other transgenes, eg. herbicide tolerance genes. More preferably, the wheat plant, grain and products therefrom are non-transgenic, i.e. they do not contain any transgene, which is preferred in some markets. Such products are also described herein as "non-transformed" products. Such non-transgenic plants and grain comprise the multiple mutant SBEII alleles as described herein, such as those produced after mutagenesis.

The terms "transgenic plant" and "transgenic wheat plant" as used herein refer to a plant that contains a gene construct ("transgene") not found in a wild-type plant of the same species, variety or cultivar. That is, transgenic plants (transformed plants) contain genetic material that they did not contain prior to the transformation. A "transgene" as referred to herein has the normal meaning in the art of biotechnology and refers to a genetic sequence which has been produced or altered by recombinant DNA or RNA technology and which has been introduced into the plant cell. The transgene may include genetic sequences obtained from or derived from a plant cell, or another plant cell, or a non-plant source, or a synthetic sequence. Typically, the transgene has been introduced into the plant by human manipulation such as, for example, by transformation but any method can be used as one of skill in the art recognizes. The genetic material is typically stably integrated into the genome of the plant. The introduced genetic material may comprise sequences that naturally occur in the same species but in a rearranged order or in a different arrangement of elements, for example an antisense sequence. Plants containing such sequences are included herein in "transgenic plants". Transgenic plants as defined herein include all progeny of an initial transformed and regenerated plant (T0 plant) which has been genetically modified using recombinant techniques, where the progeny comprise the transgene. Such progeny may be obtained by self-fertilisation of the primary transgenic plant or by crossing such plants with another plant of the same species. In an embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype. Transgenic plant parts include all parts and cells of said plants which comprise the transgene such as, for example, seeds, cultured tissues, callus and protoplasts. A "non-transgenic plant", preferably a non-transgenic wheat plant, is one which has not been genetically modified by the introduction of genetic material by recombinant DNA techniques.

As used herein, the term "corresponding non-transgenic plant" refers to a plant which is the same or similar in most characteristics, preferably isogenic or near-isogenic relative to the transgenic plant, but without the transgene of interest. Preferably, the corresponding non-transgenic plant is of the same cultivar or variety as the progenitor of the transgenic plant of interest, or a sibling plant line which lacks the construct, often termed a "segregant", or a plant of the same cultivar or variety transformed with an "empty vector" construct, and may be a non-transgenic plant. "Wild-type", as used herein, refers to a cell, tissue or plant that has not been modified according to the invention. Wild-type cells, tissue or plants known in the art and may be used as controls to compare levels of expression of an exogenous nucleic acid or the extent and nature of trait modification with cells, tissue or plants modified as described herein. As used herein, "wild-type wheat grain" means a corresponding non-mutagenized, non-transgenic wheat grain. Specific wild-type wheat grains as used herein include but are not limited to Sunstate and Cadoux. The Sunstate wheat cultivar is described in: Ellison et al., (1994) *Triticum aestivum* spp. *vulgate* (bread wheat) cv. Sunstate, *Australian Journal of Experimental Agriculture,* 34(6):869-869, the entire contents of which are incorporated herein by reference.

Any of several methods may be employed to determine the presence of a transgene in a transformed plant. For example, polymerase chain reaction (PCR) may be used to amplify sequences that are unique to the transformed plant, with detection of the amplified products by gel electrophoresis or other methods. DNA may be extracted from the plants using conventional methods and the PCR reaction carried out using primers that will distinguish the transformed and non-transformed plants. An alternative method to confirm a positive transformant is by Southern blot hybridization, well known in the art. Wheat plants which are transformed may also be identified i.e. distinguished from non-transformed or wild-type wheat plants by their phenotype, for example conferred by the presence of a selectable marker gene, or by immunoassays that detect or quantify the expression of an enzyme encoded by the transgene, or any other phenotype conferred by the transgene.

The wheat plants of the present invention may be grown or harvested for grain, primarily for use as food for human consumption or as animal feed, or for fermentation or industrial feedstock production such as ethanol production, among other uses. Alternatively, the wheat plants may be used directly as feed. The plant of the present invention is preferably useful for food production and in particular for commercial food production. Such food production might include the making of flour, dough, semolina or other products from the grain that might be an ingredient in commercial food production.

As used herein, the term "grain" generally refers to mature, harvested seed of a plant but can also refer to grain after imbibition or germination, according to the context. Mature cereal grain such as wheat commonly has a moisture content of less than about 18-20%. As used herein, the term "seed" includes harvested seed but also includes seed which is developing in the plant post anthesis and mature seed comprised in the plant prior to harvest.

As used herein, "germination" refers to the emergence of the root tip from the seed coat after imbibition. "Germination rate" refers to the percentage of seeds in a population which have germinated over a period of time, for example 7 or 10 days, after imbibition. Germination rates can be calculated using techniques known in the art. For example, a population of seeds can be assessed daily over several days to determine the germination percentage over time. With regard to grain of the present invention, as used herein the term "germination rate which is substantially the same" means that the germination rate of the grain is at least 90%, that of corresponding wild-type grain.

Starch is readily isolated from wheat grain using standard methods, for example the method of Schulman and Kammiovirta, 1991. On an industrial scale, wet or dry milling can be used Starch granule size is important in the starch processing industry where there is separation of the larger A granules from the smaller B granules.

Wild-type wheat grown commercially has a starch content in the grain which is usually in the range 55-65%, depending somewhat on the cultivar grown. In comparison, the seed or grain of the invention has a starch content of at least 90% relative to that of wild-type grain, and preferably at least 93%, at least 95%, or at least 98% relative to the starch content of wild-type grain when the plants are grown under the same conditions. In further embodiments, the starch content of the grain is at least about 25%, at least about 35%, at least about 45%, or at least about 55% to about 65% as a percentage of the grain weight (w/w). Other desirable characteristics include the capacity to mill the grain, in particular the grain hardness. Another aspect that might make a wheat plant of higher value is the degree of starch extraction from the grain, the higher extraction rates being more useful. Grain shape is also another feature that can impact on the commercial usefulness of a plant, thus grain shape can have an impact on the ease or otherwise with which the grain can be milled.

In another aspect, the invention provides starch granules or starch obtained from the grain of the plant as described above, having an increased proportion of amylose and a reduced proportion of amylopectin. Purified starch may be obtained from grain by a milling process, for example a wet milling process, which involves the separation of the starch from protein, oil and fibre. The initial product of the milling process is a mixture or composition of starch granules, and the invention therefore encompasses such granules. The starch granules from wheat comprise starch granule-bound proteins including GBSS, SBEIIa and SBEIIb amongst other proteins and therefore the presence of these proteins distinguish wheat starch granules from starch granules of other cereals. The starch from starch granules may be purified by removal of the proteins after disruption and dispersal of the starch granules by heat and/or chemical treatment. The starch granules from the wheat grain of the invention are typically distorted in shape and surface morphology, when observed under light microscopy, as exemplified herein, particularly for wheat grain having an amylose content of at least 50% as a percentage of the total starch of the grain. In an embodiment, at least 50%, preferably at least 60% or at least 70% of the starch granules obtained from the grain show distorted shape or surface morphology. The starch granules also show a loss of birefringence when observed under polarised light.

The starch of the grain, the starch of the starch granules, and the purified starch of the invention may be further characterized by one or more of the following properties:

- at least 50% (w/w), or at least 60% (w/w), or at least 67% (w/w) amylose as a proportion of the total starch;
- (ii) modified swelling volume;
- (iii) modified chain length distribution and/or branching frequency;
- (iv) modified gelatinisation temperature;
- (v) modified viscosity (peak viscosity, pasting temperature, etc.);
- (vi) modified molecular mass of amylopectin and/or amylose;
- (vii) modified % crystallinity
- (viii) comprising at least 2% resistant starch; and/or
- (ix) comprising a low relative glycaemic index (GI).

The starch may also be characterized by its swelling volume in heated excess water compared to wild-type starch. Swelling volume is typically measured by mixing either a starch or flour with excess water and heating to elevated temperatures, typically greater than 90° C. The sample is then collected by centrifugation and the swelling volume is expressed as the mass of the sedimented material divided by the dry weight of the sample. A low swelling characteristic is useful where it is desired to increase the starch content of a food preparation, in particular a hydrated food preparation.

One measure of an altered amylopectin structure is the distribution of chain lengths, or the degree of polymerization, of the starch. The chain length distribution may be determined by using fluorophore-assisted carbohydrate electrophoresis (FACE) following isoamylose de-branching. The amylopectin of the starch of the invention may have a distribution of chain length in the range from 5 to 60 that is greater than the distribution of starch from wild-type plants upon debranching. Starch with longer chain lengths will also have a commensurate decrease in frequency of branching. Thus the starch may also have a distribution of longer amylopectin chain lengths in the amylopectin still present. The amylopectin of the grain may be characterised in comprising a reduced proportion of the 4-12 dp chain length fraction relative to the amylopectin of wild-type grain, as measured after isoamylase debranching of the amylopectin.

In another aspect of the invention, the wheat starch may have an altered gelatinisation temperature, which may be readily measured by differential scanning calorimetry (DSC). Gelatinisation is the heat-driven collapse (disruption) of molecular order within the starch granule in excess water, with concomitant and irreversible changes in properties such as granular swelling, crystallite melting, loss of birefringence, viscosity development and starch solubilisation. The gelatinisation temperature may be either increased or decreased compared to starch from wild-type plants, depending on the chain length of the remaining amylopectin. High amylose starch from amylose extender (ae) mutants of maize showed a higher gelatinisation temperature than normal maize (Fuwa et al., 1999; Krueger et al., 1987). On the other hand, starch from barley sex6 mutants that lack starch synthase IIa activity had lower gelatinisation temperatures and the enthalpy for the gelatinisation peak was reduced when compared to that from control plants (Morell et al., 2003).

The gelatinisation temperature, in particular the temperature of onset of the first peak or the temperature for the apex of the first peak, may be elevated by at least 3° C., preferably at least 5° C. or more preferably at least 7° C. as measured by DSC compared to starch extracted from a similar, but unal-

tered grain. The starch may comprise an elevated level of resistant starch, with an altered structure indicated by specific physical characteristics including one or more of the group consisting of physical inaccessibility to digestive enzymes which may be by reason of having altered starch granule morphology, the presence of appreciable starch associated lipid, altered crystallinity, and altered amylopectin chain length distribution. The high proportion of amylose also contributes to the level of resistant starch.

The starch structure of the wheat of the present invention may also differ in that the degree of crystallinity is reduced compared to normal starch isolated from wheat. The reduced crystallinity of a starch is also thought to be associated with enhance organoleptic properties and contributes to a smoother mouth feel. Thus, the starch may additionally exhibit reduced crystallinity resulting from reduced levels of activity of one or more amylopectin synthesis enzymes. Crystallinity is typically investigated by X-ray crystallography.

In some embodiments, the present starch provides modified digestive properties such as increased resistant starch including between 1% to 20%, 2% to 18%, 3% to 18% or 5% to 15% resistant starch and a decreased Glycaemic Index (GI).

The invention also provides flour, meal or other products produced from the grain. These may be unprocessed or processed, for example by fractionation or bleaching.

The invention also provides starch from grain of the exemplified wheat plants comprising increased amounts of dietary fibre, preferably in combination with an elevated level of resistant starch. This increase is also at least in part a result of the high relative level of amylose.

The term "dietary fibre" as used herein includes the carbohydrate and carbohydrate digestion products which are not absorbed in the small intestine of healthy humans but which enter the large bowel. This includes resistant starch and other soluble and insoluble carbohydrate polymers. It is intended to comprise that portion of carbohydrates that are fermentable, at least partially, in the large bowel by the resident microflora. The starch of the invention contains relatively high levels of dietary fibre, more particularly amylose. The dietary fibre content of the grain of the present invention results at least in part from the increased amylose content in the starch of the grain, and also, or in combination with an increased resistant starch content as a percentage of the total starch. "Resistant starch" is defined herein as the sum of starch and products of starch digestion not absorbed in the small intestine of healthy humans but entering into the large bowel. This is defined in terms of a percentage of the total starch of the grain, or a percentage of the total starch content in the food, according to the context. Thus, resistant starch excludes products digested and absorbed in the small intestine. Resistant starches include physically inaccessible starch (RS1 form), resistant native starch granules (RS2), retrograded starches (RS3), and chemically modified starches (RS4). The altered starch structure and in particular the high amylose levels of the starch of the invention give rise to an increase in resistant starch when consumed in food. The starch may be in an RS1 form, being somewhat inaccessible to digestion. Starch-lipid association as measured by V-complex crystallinity is also likely to contribute to the level of resistant starch.

Whilst the invention may be particularly useful in the treatment or prophylaxis of humans, it is to be understood that the invention is also applicable to non-human subjects including but not limited to agricultural animals such as cows, sheep, pigs and the like, domestic animals such as dogs or cats, laboratory animals such as rabbits or rodents such as mice, rats, hamsters, or animals that might be used for sport such as horses. The method may be particularly applicable to non-ruminant mammals or animals such as mono-gastric mammals. The invention may also be applicable to other agricultural animals for example poultry including, for example, chicken, geese, ducks, turkeys, or quails, or fish.

The method of treating the subject, particularly humans, may comprise the step of administering altered wheat grain, flour, starch or a food or drink product as defined herein to the subject, in one or more doses, in an amount and for a period of time whereby the level of the one or more of the bowel health or metabolic indicators improves. The indicator may change relative to consumption of non-altered wheat starch or wheat or product thereof, within a time period of hours, as in the case of some of the indicators such as pH, elevation of levels of SCFA, post-prandial glucose fluctuation, or it may take days such as in the case of increase in fecal bulk or improved laxation, or perhaps longer in the order of weeks or months such as in the case where the butyrate enhanced proliferation of normal colonocytes is measured. It may be desirable that administration of the altered starch or wheat or wheat product be lifelong. However, there are good prospects for compliance by the individual being treated given the relative ease with which the altered starch can be administered.

Dosages may vary depending on the condition being treated or prevented but are envisaged for humans as being at least 1 g of wheat grain or starch of the invention per day, more preferably at least 2 g per day, preferably at least 10 or at least 20 g per day. Administration of greater than about 100 grams per day may require considerable volumes of delivery and reduce compliance. Most preferably the dosage for a human is between 5 and 60 g of wheat grain or starch per day, or for adults between 5 and 100 g per day.

Glycaemic Index (GI) relates to the rate of digestion of foods comprising the starch, and is a comparison of the effect of a test food with the effect of white bread or glucose on excursions in blood glucose concentration. The Glycaemic Index is a measure of the likely effect of the food concerned on post prandial serum glucose concentration and demand for insulin for blood glucose homeostasis. One important characteristic provided by foods of the invention is a reduced glycaemic index. Serum glucose levels were lower 30 min after ingestion of high amylose wheat products by human volunteers compared to low amylose wheat (Goddard et al., 1984). Furthermore, the foods may have a low level of final digestion and consequently be relatively low-calorie. A low calorific product might be based on inclusion of flour produced from milled wheat grain. Such foods may have the effect of being filling, enhancing bowel health, reducing the post-prandial serum glucose and lipid concentration as well as providing for a low calorific food product.

The indicators of improved bowel health may comprise, but are not necessarily limited to:

i) decreased pH of the bowel contents,
ii) increased total SCFA concentration or total SCFA amount in the bowel contents,
iii) increased concentration or amount of one or more SCFAs contents,
iv) increased fecal bulk,
v) increase in total water volume of bowel or faeces, without diarrhea,
vi) improved laxation,
vii) increase in number or activity of one or more species of probiotic bacteria,
viii) increase in fecal bile acid excretion,
ix) reduced urinary levels of putrefactive products,
x) reduced fecal levels of putrefactive products,
xi) increased proliferation of normal colonocytes,

xii) reduced inflammation in the bowel of individuals with inflamed bowel,

xiii) reduced fecal or large bowel levels of any one of urea, creatinine and phosphate in uremic patients, and

xiv) any combination of the above.

The indicators of improved metabolic health may comprise, but are not necessarily limited to:

i) stabilisation of post-prandial glucose fluctuation,

ii) improved (lowered) glycaemic response,

iii) reduced pro-prandial plasma insulin concentration,

iv) improved blood lipid profile,

v) lowering of plasma LDL cholesterol,

vi) reduced plasma levels of one or more of urea, creatinine and phosphate in uremic patients,

vii) an improvement in a dysglucaemic response, or

viii) any combination of the above.

It will be understood that one benefit of the present invention is that it provides for products such as bread that are of particular nutritional benefit, and moreover it does so without the need to post-harvest modify the starch or other constituents of the wheat grain. However, it may be desired to make modifications to the starch or other constituent of the grain, and the invention encompasses such a modified constituent. Methods of modification are well known and include the extraction of the starch or other constituent by conventional methods and modification of the starches to increase the resistant form. The starch may be modified by treatment with heat and/or moisture, physically (for example ball milling), enzymatically (using for example α- or β-amylase, pullalanase or the like), chemical hydrolysis (wet or dry using liquid or gaseous reagents), oxidation, cross bonding with difunctional reagents (for example sodium trimetaphosphate, phosphorus oxychloride), or carboxymethylation.

The wheat starch of the present invention will be a suitable substrate for fermentation for ethanol (biofuel) or ethanol-containing beverages and the wheat grain or wheat starch for other fermentation products such as foods, nutraceuticals (insoluble or soluble fibre), enzymes and industrial materials. The methods for fermentation using plant-derived starch are well known to those skilled in the art, with established processes for various fermentation products (see for example Vogel et al., 1996 and references cited therein). In one embodiment, the starch carbohydrates may be extracted by crushing the wheat plant parts of the invention such as grain, or by diffusion from the plant tissues into water or another suitable solvent. Wheat tissues or starch of the invention may be used directly as a substrate for fermentation or bioconversion in a batch, continuous, or immobilized-cell process.

The terms "polypeptide" and "protein" are generally used interchangeably herein. The terms "proteins" and "polypeptides" as used herein also include variants, mutants, modifications and/or derivatives of the polypeptides of the invention as described herein. As used herein, "substantially purified polypeptide" refers to a polypeptide that has been separated from the lipids, nucleic acids, other peptides and other molecules with which it is associated in its native state. Preferably, the substantially purified polypeptide is at least 60% free, more preferably at least 75% free, and more preferably at least 90% free from other components with which it is naturally associated. By "recombinant polypeptide" is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant polynucleotide in a cell, preferably a plant cell and more preferably a wheat cell. In an embodiment, the polypeptide has starch branching enzyme activity, particularly SBEII activity, and is at least 90% identical to a SBEII described herein.

As used herein a "biologically active" fragment is a portion of a polypeptide of the invention which maintains a defined activity of the full-length polypeptide. In a particularly preferred embodiment, the biologically active fragment has starch branching enzyme activity. Biologically active fragments can be any size as long as they maintain the defined activity, but are preferably at least 700 or 800 amino acid residues long.

The % identity of a polypeptide relative to another polypeptide can be determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 50 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 50 amino acids. More preferably, the query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. Even more preferably, the query sequence is at least 250 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 250 amino acids. Most preferably, two SBEII polypeptides are aligned over their full length amino acid sequences.

With regard to a defined polypeptide, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide comprises an amino acid sequence which is at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

Amino acid sequence mutants of the polypeptides of the present invention can be prepared by introducing appropriate nucleotide changes into a nucleic acid of the present invention or by mutagenesis in vivo such as by chemical or radiation treatment. Such mutants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. The polynucleotides of the invention may be subjected to DNA shuffling techniques as described by Harayama, 1998 or other in vitro methods to produce altered polynucleotides which encode polypeptide variants. These DNA shuffling techniques may use genetic sequences related to those of the present invention, such as SBE genes from plant species other than wheat. Products derived from mutated/altered DNA can readily be screened using techniques described herein to determine if they possess, for example, SBE activity.

Amino acid sequence deletions generally range from about 1 to 15 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various strains or species are identical i.e. conserved amino acids. These positions may be important for biological activity. These amino acids, especially those falling within a contiguous sequence of at least three other

identically conserved amino acids, are preferably substituted in a relatively conservative manner in order to retain function such as SBEII activity. Such conservative substitutions are shown in Table 1 under the heading of "exemplary substitutions". "Non-conservative amino acid substitutions" are defined herein as substitutions other than those listed in Table 3 (Exemplary conservative substitutions). Non-conservative substitutions in an SBEII are expected to reduce the activity of the enzyme and many will correspond to an SBEII encoded by a "partial loss of function mutant SBEII gene".

Also included within the scope of the invention are polypeptides of the present invention which are differentially modified during or after synthesis, e.g., by phosphorylation, as has been shown for SBEI, SBEIIa and SBEIIb in amyloplasts of wheat (Tetlow et al., 2004). These modifications may serve to regulate the activity of the enzyme, for example by regulating the formation of protein complexes in amyloplasts during starch synthesis (Tetlow et al., 2008), or to increase the stability and/or bioactivity of the polypeptide of the invention, or serve as a ligand for binding of another molecule.

In some embodiments, the present invention involves modification of gene activity, particularly of SBEII gene activity, combinations of mutant genes, and the construction and use of chimeric genes. As used herein, the term "gene" includes any deoxyribonucleotide sequence which includes a protein coding region or which is transcribed in a cell but not translated, together with associated non-coding and regulatory regions. Such associated regions are typically located adjacent to the coding region on both the 5 and 3' ends for a distance of about 2 kb on either side. In this regard, the gene includes control signals such as promoters, enhancers, transcription termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control signals in which case the gene is referred to as a "chimeric gene". The sequences which are located 5' of the protein coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the protein coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. The term "gene" includes synthetic or fusion molecules encoding the proteins of the invention described herein. Genes are ordinarily present in the wheat genome as double-stranded DNA. A chimeric gene may be introduced into an appropriate vector for extrachromosomal maintenance in a cell or for integration into the host genome. Genes or genotypes as referred to herein in italicised form (e.g. SBEIIa) while proteins, enzymes or phenotypes are referred to in non-italicised form (SBEIIa).

A genomic form or clone of a gene containing the coding region may be interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." An "intron" as used herein is a segment of a gene which is transcribed as part of a primary RNA transcript but is not present in the mature mRNA molecule. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA). Introns may contain regulatory elements such as enhancers. "Exons" as used herein refer to the DNA regions corresponding to the RNA sequences which are present in the mature mRNA or the mature RNA molecule in cases where the RNA molecule is not translated. An mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

The present invention refers to various polynucleotides. As used herein, a "polynucleotide" or "nucleic acid" or "nucleic acid molecule" means a polymer of nucleotides, which may be DNA or RNA or a combination thereof, for example a heteroduplex of DNA and RNA, and includes for example mRNA, cRNA, cDNA, tRNA, siRNA, shRNA, hpRNA, and single or double-stranded DNA. It may be DNA or RNA of cellular, genomic or synthetic origin, for example made on an automated synthesizer, and may be combined with carbohydrate, lipids, protein or other materials, labelled with fluorescent or other groups, or attached to a solid support to perform a particular activity defined herein. Preferably the polynucleotide is solely DNA or solely RNA as occurs in a cell, and some bases may be methylated or otherwise modified as occurs in a wheat cell. The polymer may be single-stranded, essentially double-stranded or partly double-stranded. An example of a partly-double stranded RNA molecule is a hairpin RNA (hpRNA), short hairpin RNA (shRNA) or self-complementary RNA which include a double stranded stem formed by basepairing between a nucleotide sequence and its complement and a loop sequence which covalently joins the nucleotide sequence and its complement. Basepairing as used herein refers to standard basepairing between nucleotides, including G:U basepairs in an RNA molecule. "Complementary" means two polynucleotides are capable of basepairing along part of their lengths, or along the full length of one or both.

By "isolated" is meant material that is substantially or essentially free from components that normally accompany it in its native state. As used herein, an "isolated polynucleotide" or "isolated nucleic acid molecule" means a polynucleotide which is at least partially separated from, preferably substantially or essentially free of, the polynucleotide sequences of the same type with which it is associated or linked in its native state. For example, an "isolated polynucleotide" includes a polynucleotide which has been purified or separated from the sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment. Preferably, the isolated polynucleotide is also at least 90% free from other components such as proteins, carbohydrates, lipids etc. The term "recombinant polynucleotide" as used herein refers to a polynucleotide formed in vitro by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably connected to the nucleotide sequence to be transcribed in the cell.

The present invention refers to use of oligonucleotides which may be used as "probes" or "primers". As used herein, "oligonucleotides" are polynucleotides up to 50 nucleotides in length. They can be RNA, DNA, or combinations or derivatives of either. Oligonucleotides are typically relatively short single stranded molecules of 10 to 30 nucleotides, commonly 15-25 nucleotides in length, typically comprised of 10-30 or 15-25 nucleotides which are identical to, or complementary to, part of an SBEIIa or SBEIIb gene or cDNA corresponding to an SBEIIa or SBEIIb gene. When used as a probe or as a primer in an amplification reaction, the minimum size of such an oligonucleotide is the size required for the formation of a stable hybrid between the oligonucleotide and a complementary sequence on a target nucleic acid molecule. Preferably, the oligonucleotides are at least 15 nucleotides, more preferably at least 18 nucleotides, more preferably at least 19 nucleotides, more preferably at least 20 nucleotides, even more preferably at least 25 nucleotides in length. Polynucleotides used as a probe are typically conjugated with a detectable label such as a radioisotope, an enzyme, biotin, a fluorescent

molecule or a chemiluminescent molecule. Oligonucleotides and probes of the invention are useful in methods of detecting an allele of a SBEIIa, SBEIIb or other gene associated with a trait of interest, for example modified starch. Such methods employ nucleic acid hybridization and in many instances include oligonucleotide primer extension by a suitable polymerase, for example as used in PCR for detection or identification of wild-type or mutant alleles. Preferred oligonucleotides and probes hybridise to a SBEIIa or SBEIIb gene sequence from wheat, including any of the sequences disclosed herein, for example SEQ ID NOs: 36 to 149. Preferred oligonucleotide pairs are those that span one or more introns, or a part of an intron and therefore may be used to amplify an intron sequence in a PCR reaction. Numerous examples are provided in the Examples herein.

The terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence and which are able to function in an analogous manner to, or with the same activity as, the reference sequence. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide, or that have, when compared to naturally occurring molecules, one or more mutations. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. Accordingly, these terms encompass polynucleotides that encode polypeptides that exhibit enzymatic or other regulatory activity, or polynucleotides capable of serving as selective probes or other hybridising agents. The terms "polynucleotide variant" and "variant" also include naturally occurring allelic variants. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the nucleic acid). Preferably, a polynucleotide variant of the invention which encodes a polypeptide with enzyme activity is greater than 400, more preferably greater than 500, more preferably greater than 600, more preferably greater than 700, more preferably greater than 800, more preferably greater than 900, and even more preferably greater than 1,000 nucleotides in length, up to the full length of the gene.

A variant of an oligonucleotide of the invention includes molecules of varying sizes which are capable of hybridising, for example, to the wheat genome at a position close to that of the specific oligonucleotide molecules defined herein. For example, variants may comprise additional nucleotides (such as 1, 2, 3, 4, or more), or less nucleotides as long as they still hybridise to the target region. Furthermore, a few nucleotides may be substituted without influencing the ability of the oligonucleotide to hybridise to the target region. In addition, variants may readily be designed which hybridise close (for example, but not limited to, within 50 nucleotides) to the region of the plant genome where the specific oligonucleotides defined herein hybridise.

By "corresponds to" or "corresponding to" in the context of polynucleotides or polypeptides is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein. Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", "substantial identity" and "identical", and are defined with respect to a defined minimum number of nucleotides or amino acid residues or preferably over the full length. The terms "sequence identity" and "identity" are used interchangeably herein to refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The % identity of a polynucleotide can be determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Unless stated otherwise, the query sequence is at least 45 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 45 nucleotides. Preferably, the query sequence is at least 150 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 150 nucleotides. More preferably, the query sequence is at least 300 nucleotides in length and the GAP analysis aligns the two sequences over a region of at least 300 nucleotides, or at least 400, 500 or 600 nucleotides in each case. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1997. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel et al., 1994-1998, Chapter 15.

Nucleotide or amino acid sequences are indicated as "essentially similar" when such sequences have a sequence identity of at least about 95%, particularly at least about 98%, more particularly at least about 98.5%, quite particularly about 99%, especially about 99.5%, more especially about 100%, quite especially are identical. It is clear that when RNA sequences are described as essentially similar to, or have a certain degree of sequence identity with, DNA sequences, thymine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence.

With regard to the defined polynucleotides, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polynucleotide comprises a polynucleotide sequence which is at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably

at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

In some embodiments, the present invention refers to the stringency of hybridization conditions to define the extent of complementarity of two polynucleotides. "Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between a target nucleotide sequence and the labelled polynucleotide sequence. "Stringent conditions" refers to temperature and ionic conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridize. As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, herein incorporated by reference. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6× sodium chloride/sodium citrate (SSC) at about 45° C., followed by two washes in 0.2×SSC, 0.1% SDS at 50-55° C.; 2) medium stringency hybridization conditions in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 60° C.; 3) high stringency hybridization conditions in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.; and 4) very high stringency hybridization conditions are 0.5 M sodium phosphate, 7% SDS at 65° C., followed by one or more washes at 0.2×SSC, 1% SDS at 65° C.

As used herein, a "chimeric gene" or "genetic construct" refers to any gene that is not a native gene in its native location i.e. it has been artificially manipulated, including a chimeric gene or genetic construct which is integrated into the wheat genome. Typically a chimeric gene or genetic construct comprises regulatory and transcribed or protein coding sequences that are not found together in nature. Accordingly, a chimeric gene or genetic construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. The term "endogenous" is used herein to refer to a substance that is normally produced in an unmodified plant at the same developmental stage as the plant under investigation, preferably a wheat plant, such as starch or a SBEIIa or SBEIIb. An "endogenous gene" refers to a native gene in its natural location in the genome of an organism, preferably a SBEIIa or SBEIIb gene in a wheat plant. As used herein, "recombinant nucleic acid molecule" refers to a nucleic acid molecule which has been constructed or modified by recombinant DNA technology. The terms "foreign polynucleotide" or "exogenous polynucleotide" or "heterologous polynucleotide" and the like refer to any nucleic acid which is introduced into the genome of a cell by experimental manipulations, preferably the wheat genome, but which does not naturally occur in the cell. These include modified forms of gene sequences found in that cell so long as the introduced gene contains some modification, e.g. an introduced mutation or the presence of a selectable marker gene, relative to the naturally-occurring gene. Foreign or exogenous genes may be genes found in nature that are inserted into a non-native organism, native genes introduced into a new location within the native host, or chimeric genes or genetic constructs. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The term "genetically modified" includes introducing genes into cells, mutating genes in cells and altering or modulating the regulation of a gene in a cell or organisms to which these acts have been done or their progeny.

The present invention refers to elements which are operably connected or linked. "Operably connected" or "operably linked" and the like refer to a linkage of polynucleotide elements in a functional relationship. Typically, operably connected nucleic acid sequences are contiguously linked and, where necessary to join two protein coding regions, contiguous and in reading frame. A coding sequence is "operably connected to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single RNA, which if translated is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences are ultimately processed to produce the desired protein.

As used herein, the term "cis-acting sequence", "cis-acting element" or "cis-regulatory region" or "regulatory region" or similar term shall be taken to mean any sequence of nucleotides which regulates the expression of the genetic sequence. This may be a naturally occurring cis-acting sequence in its native context, for example regulating a wheat SBEIIa or SBEIIb gene, or a sequence in a genetic construct which when positioned appropriately relative to an expressible genetic sequence, regulates its expression. Such a cis-regulatory region may be capable of activating, silencing, enhancing, repressing or otherwise altering the level of expression and/or cell-type-specificity and/or developmental specificity of a gene sequence at the transcriptional or post-transcriptional level. In preferred embodiments of the present invention, the cis-acting sequence is an activator sequence that enhances or stimulates the expression of an expressible genetic sequence, such as a promoter. The presence of an intron in the 5'-leader (UTR) of genes has been shown to enhance expression of genes in monocotyledonous plants such as wheat (Tanaka et al., 1990). Another type of cis-acting sequence is a matrix attachment region (MAR) which may influence gene expression by anchoring active chromatin domains to the nuclear matrix.

"Operably connecting" a promoter or enhancer element to a transcribable polynucleotide means placing the transcribable polynucleotide (e.g., protein-encoding polynucleotide or other transcript) under the regulatory control of a promoter, which then controls the transcription of that polynucleotide. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position a promoter or variant thereof at a distance from the transcription start site of the transcribable polynucleotide, which is approximately the same as the distance between that promoter and the gene it controls in its natural setting; i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function.

The present invention makes use of vectors for production, manipulation or transfer of chimeric genes or genetic constructs. By "vector" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage or plant virus, into which a nucleic acid sequence may be inserted. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable into the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is indepen-

dent of chromosomal replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into a cell, is integrated into the genome of the recipient cell and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants, or sequences that enhance transformation of prokaryotic or eukaryotic (especially wheat) cells such as T-DNA or P-DNA sequences. Examples of such resistance genes and sequences are well known to those of skill in the art.

By "marker gene" is meant a gene that imparts a distinct phenotype to cells expressing the marker gene and thus allows such transformed cells to be distinguished from cells that do not have the marker. A "selectable marker gene" confers a trait for which one can 'select' based on resistance to a selective agent (e.g., a herbicide, antibiotic, radiation, heat, or other treatment damaging to untransformed cells) or based on a growth advantage in the presence of a metabolizable substrate. A screenable marker gene (or reporter gene) confers a trait that one can identify through observation or testing, i.e., by 'screening' (e.g., β-glucuronidase, luciferase, GFP or other enzyme activity not present in untransformed cells). The marker gene and the nucleotide sequence of interest do not have to be linked.

Examples of bacterial selectable markers are markers that confer antibiotic resistance such as ampicillin, kanamycin, erythromycin, chloramphenicol or tetracycline resistance. Exemplary selectable markers for selection of plant transformants include, but are not limited to, a hyg gene which confers hygromycin B resistance; a neomycin phosphotransferase (npt) gene conferring resistance to kanamycin, paromomycin, G418 and the like as, for example, described by Potrykus et al., 1985; a glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides as, for example, described in EP-A-256223; a glutamine synthetase gene conferring, upon overexpression, resistance to glutamine synthetase inhibitors such as phosphinothricin as, for example, described WO87/05327, an acetyl transferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin as, for example, described in EP-A-275957, a gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine as, for example, described by Hinchee et al., 1988, a bar gene conferring resistance against bialaphos as, for example, described in WO91/02071; a nitrilase gene such as bxn from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker et al. 1988); a dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate (Thillet et al, 1988); a mutant acetolactate synthase gene (ALS), which confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (EP-A-154204); a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; or a dalapon dehalogenase gene that confers resistance to the herbicide.

Preferred screenable markers include, but are not limited to, a uidA gene encoding a β-glucuronidase (GUS) enzyme for which various chromogenic substrates are known, a β-galactosidase gene encoding an enzyme for which chromogenic substrates are known, an aequorin gene (Prasher et al., 1985), which may be employed in calcium-sensitive bioluminescence detection; a green fluorescent protein gene (GFP, Niedz et al., 1995) or one of its variants; a luciferase (luc) gene (Ow et al., 1986), which allows for bioluminescence detection, and others known in the art.

In some embodiments, the level of endogenous starch branching activity or other enzyme activity is modulated by decreasing the level of expression of genes encoding proteins involved in these activities in the wheat plant, or increasing the level of expression of a nucleotide sequence that codes for the enzyme in a wheat plant. Increasing expression can be achieved at the level of transcription by using promoters of different strengths or inducible promoters, which are capable of controlling the level of transcript expressed from the coding sequence. Heterologous sequences may be introduced which encode transcription factors that modulate or enhance expression of genes whose products down regulate starch branching. The level of expression of the gene may be modulated by altering the copy number per cell of a construct comprising the coding sequence and a transcriptional control element that is operably connected thereto and that is functional in the cell. Alternatively, a plurality of transformants may be selected, and screened for those with a favourable level and/or specificity of transgene expression arising from influences of endogenous sequences in the vicinity of the transgene integration site. A favourable level and pattern of transgene expression is one which results in a substantial increase in starch synthesis or amylose content in the wheat plant. This may be detected by simple testing of transformants.

Reducing gene expression may be achieved through introduction and transcription of a "gene-silencing chimeric gene" introduced into the wheat plant. The gene-silencing chimeric gene is preferably introduced stably into the wheat genome, preferably the wheat nuclear genome. As used herein "gene-silencing effect" refers to the reduction of expression of a target nucleic acid in a wheat cell, preferably an endosperm cell, which can be achieved by introduction of a silencing RNA. In a preferred embodiment, a gene-silencing chimeric gene is introduced which encodes an RNA molecule which reduces expression of one or more endogenous genes, preferably the SBEIIa and/or SBEIIb genes. Target genes in wheat also include the genes listed in Table 1. Such reduction may be the result of reduction of transcription, including via methylation of chromatin remodeling, or post-transcriptional modification of the RNA molecules, including via RNA degradation, or both. Gene-silencing should not necessarily be interpreted as an abolishing of the expression of the target nucleic acid or gene. It is sufficient that the level expression of the target nucleic acid in the presence of the silencing RNA is lower that in the absence thereof. The level of expression of the targeted gene may be reduced by at least about 40% or at least about 45% or at least about 50% or at least about 55% or at least about 60% or at least about 65% or at least about 70% or at least about 75% or at least about 80% or at least about 85% or at least about 90% or at least about 95% or effectively abolished to an undetectable level.

Antisense techniques may be used to reduce gene expression in wheat cells. The term "antisense RNA" shall be taken to mean an RNA molecule that is complementary to at least a portion of a specific mRNA molecule and capable of reducing expression of the gene encoding the mRNA, preferably a SBEIIa and/or SBEIIb gene. Such reduction typically occurs in a sequence-dependent manner and is thought to occur by interfering with a post-transcriptional event such as mRNA

transport from nucleus to cytoplasm, mRNA stability or inhibition of translation. The use of antisense methods is well known in the art (see for example, Hartmann and Endres, 1999). Antisense methods are now a well established technique for manipulating gene expression in plants.

Antisense molecules typically include sequences that correspond to part of the transcribed region of a target gene or for sequences that effect control over the gene expression or splicing event. For example, the antisense sequence may correspond to the targeted protein coding region of the genes of the invention, or the 5'-untranslated region (UTR) or the 3'-UTR or combination of these, preferably only to exon sequences of the target gene. In view of the generally greater divergence between related genes of the UTRs, targeting these regions provides greater specificity of gene inhibition. The length of the antisense sequence should be at least 19 contiguous nucleotides, preferably at least 50 nucleotides, and more preferably at least 100, 200, 500 or 1000 nucleotides, to a maximum of the full length of the gene to be inhibited. The full-length sequence complementary to the entire gene transcript may be used. The length is most preferably 100-2000 nucleotides. The degree of identity of the antisense sequence to the targeted transcript should be at least 90% and more preferably 95-100%. The antisense RNA molecule may of course comprise unrelated sequences which may function to stabilize the molecule.

Genetic constructs to express an antisense RNA may be readily made by joining a promoter sequence to a region of the target gene in an "antisense" orientation, which as used herein refers to the reverse orientation relative to the orientation of transcription and translation (if it occurs) of the sequence in the target gene in the plant cell. Preferably, the antisense RNA is expressed preferentially in the endosperm of a wheat plant by use of an endosperm-specific promoter.

The term "ribozyme" refers to an RNA molecule which specifically recognizes a distinct substrate RNA and catalyzes its cleavage. Typically, the ribozyme contains an antisense sequence for specific recognition of a target nucleic acid, and an enzymatic region referred to herein as the "catalytic domain". The types of ribozymes that are particularly useful in this invention are the hammerhead ribozyme (Haseloff and Gerlach, 1988; Perriman et al., 1992) and the hairpin ribozyme (Shippy et al., 1999).

As used herein, "artificially introduced dsRNA molecule" refers to the introduction of double-stranded RNA (dsRNA) molecule, which preferably is synthesised in the wheat cell by transcription from a chimeric gene encoding such dsRNA molecule. RNA interference (RNAi) is particularly useful for specifically reducing the expression of a gene or inhibiting the production of a particular protein, also in wheat (Regina et al., 2006). This technology relies on the presence of dsRNA molecules that contain a sequence that is essentially identical to the mRNA of the gene of interest or part thereof, and its complement, thereby forming a dsRNA. Conveniently, the dsRNA can be produced from a single promoter in the host cell, where the sense and anti-sense sequences are transcribed to produce a hairpin RNA in which the sense and anti-sense sequences hybridize to form the dsRNA region with a related (to a SBEII gene) or unrelated sequence forming a loop structure, so the hairpin RNA comprises a stem-loop structure. The design and production of suitable dsRNA molecules for the present invention is well within the capacity of a person skilled in the art, particularly considering Waterhouse et al., 1998; Smith et al., 2000; WO 99/32619; WO 99/53050; WO 99/49029; and WO 01/34815.

The DNA encoding the dsRNA typically comprises both sense and antisense sequences arranged as an inverted repeat. In a preferred embodiment, the sense and antisense sequences are separated by a spacer region that comprises an intron which, when transcribed into RNA, is spliced out. This arrangement has been shown to result in a higher efficiency of gene silencing (Smith et al., 2000). The double-stranded region may comprise one or two RNA molecules, transcribed from either one DNA region or two. The dsRNA may be classified as long hpRNA, having long, sense and antisense regions which can be largely complementary, but need not be entirely complementary (typically larger than about 200 bp, ranging between 200-1000 bp). hpRNA can also be rather small with the double-stranded portion ranging in size from about 30 to about 42 bp, but not much longer than 94 bp (see WO04/073390). The presence of the double stranded RNA region is thought to trigger a response from an endogenous plant system that destroys both the double stranded RNA and also the homologous RNA transcript from the target plant gene, efficiently reducing or eliminating the activity of the target gene.

The length of the sense and antisense sequences that hybridise should each be at least 19 contiguous nucleotides, preferably at least 30 or 50 nucleotides, and more preferably at least 100, 200, 500 or 1000 nucleotides. The full-length sequence corresponding to the entire gene transcript may be used. The lengths are most preferably 100-2000 nucleotides. The degree of identity of the sense and antisense sequences to the targeted transcript should be at least 85%, preferably at least 90% and more preferably 95-100%. The longer the sequence, the less stringent the requirement for the overall sequence identity. The RNA molecule may of course comprise unrelated sequences which may function to stabilize the molecule. The promoter used to express the dsRNA-forming construct may be any type of promoter that is expressed in the cells which express the target gene. When the target gene is SBEIIa or SBEIIb or other gene expressed selectively in the endosperm, an endosperm promoter is preferred, so as to not affect expression of the target gene(s) in other tissues.

Examples of dsRNA molecules that may be used to down-regulate SBEII gene(s) are provided in Example 4.

Other silencing RNA may be "unpolyadenylated RNA" comprising at least 20 consecutive nucleotides having at least 95% sequence identity to the complement of a nucleotide sequence of an RNA transcript of the target gene, such as described in WO01/12824 or U.S. Pat. No. 6,423,885. Yet another type of silencing RNA is an RNA molecule as described in 003/076619 (herein incorporated by reference) comprising at least 20 consecutive nucleotides having at least 95% sequence identity to the sequence of the target nucleic acid or the complement thereof, and further comprising a largely-double stranded region as described in WO03/076619.

As used herein, "silencing RNAs" are RNA molecules that have 21 to 24 contiguous nucleotides that are complementary to a region of the mRNA transcribed from the target gene, preferably SBEIIa or SBEIIb. The sequence of the 21 to 24 nucleotides is preferably fully complementary to a sequence of 21 to 24 contiguous nucleotides of the mRNA i.e. identical to the complement of the 21 to 24 nucleotides of the region of the mRNA. However, miRNA sequences which have up to five mismatches in region of the mRNA may also be used (Palatnik et al., 2003), and basepairing may involve one or two G-U basepairs. When not all of the 21 to 24 nucleotides of the silencing RNA are able to basepair with the mRNA, it is preferred that there are only one or two mismatches between the 21 to 24 nucleotides of the silencing RNA and the region of the mRNA. With respect to the miRNAs, it is preferred that any mismatches, up to the maximum of five, are

found towards the 3' end of the miRNA. In a preferred embodiment, there are not more than one or two mismatches between the sequences of the silencing RNA and its target mRNA.

Silencing RNAs derive from longer RNA molecules that are encoded by the chimeric DNAs of the invention. The longer RNA molecules, also referred to herein as "precursor RNAs", are the initial products produced by transcription from the chimeric DNAs in the wheat cells and have partially double-stranded character formed by intra-molecular base-pairing between complementary regions. The precursor RNAs are processed by a specialized class of RNAses, commonly called "Dicer(s)", into the silencing RNAs, typically of 21 to 24 nucleotides long. Silencing RNAs as used herein include short interfering RNAs (siRNAs) and microRNAs (miRNAs), which differ in their biosynthesis. SiRNAs derive from fully or partially double-stranded RNAs having at least 21 contiguous basepairs, including possible G-U basepairs, without mismatches or non-basepaired nucleotides bulging out from the double-stranded region. These double-stranded RNAs are formed from either a single, self-complementary transcript which forms by folding back on itself and forming a stem-loop structure, referred to herein as a "hairpin RNA", or from two separate RNAs which are at least partly complementary and that hybridize to form a double-stranded RNA region. mRNAs are produced by processing of longer, single-stranded transcripts that include complementary regions that are not fully complementary and so form an imperfectly basepaired structure, so having mismatched or non-basepaired nucleotides within the partly double-stranded structure. The basepaired structure may also include G-U basepairs. Processing of the precursor RNAs to form miRNAs leads to the preferential accumulation of one distinct, small RNA having a specific sequence, the miRNA. It is derived from one strand of the precursor RNA, typically the "antisense" strand of the precursor RNA, whereas processing of the long complementary precursor RNA to form siRNAs produces a population of siRNAs which are not uniform in sequence but correspond to many portions and from both strands of the precursor. mRNAs were first discovered as a small regulatory RNA controlling the lin-4 gene in *C. elegans* (Lee et al., 1993). Since then, large numbers of other naturally occurring miRNAs have been reported to be involved in regulation of gene function in animals and plants. mRNA precursor RNAs of the invention, also termed herein as "artificial miRNA precursors", are typically derived from naturally occurring miRNA precursors by altering the nucleotide sequence of the miRNA portion of the naturally-occurring precursor so that it is complementary, preferably fully complementary, to the 21 to 24 nucleotide region of the target mRNA, and altering the nucleotide sequence of the complementary region of the miRNA precursor that basepairs to the miRNA sequence to maintain basepairing. The remainder of the miRNA precursor RNA may be unaltered and so have the same sequence as the naturally occurring miRNA precursor, or it may also be altered in sequence by nucleotide substitutions, nucleotide insertions, or preferably nucleotide deletions, or any combination thereof. The remainder of the miRNA precursor RNA is thought to be involved in recognition of the structure by the Dicer enzyme called Dicer-like 1 (DCL1), and therefore it is preferred that few if any changes are made to the remainder of the structure. For example, basepaired nucleotides may be substituted for other basepaired nucleotides without major change to the overall structure. The naturally occurring miRNA precursor from which the artificial miRNA precursor of the invention is derived may be from wheat, another plant such as another cereal plant, or from non-plant sources. Examples of such precursor RNAs are the rice mi395 precursor, the *Arabidopsis* mi159b precursor, or the mi172 precursor.

Artificial miRNAs have been demonstrated in plants, for example Alvarez et al., 2006; Parizotto et al., 2004; Schwab et al., 2006.

Another molecular biological approach that may be used is co-suppression. The mechanism of co-suppression is not well understood but is thought to involve post-transcriptional gene silencing (PTGS) and in that regard may be very similar to many examples of antisense suppression. It involves introducing an extra copy of a gene or a fragment thereof into a plant in the "sense orientation" with respect to a promoter for its expression, which as used herein refers to the same orientation as transcription and translation (if it occurs) of the sequence relative to the sequence in the target gene. The size of the sense fragment, its correspondence to target gene regions, and its degree of homology to the target gene are as for the antisense sequences described above. In some instances the additional copy of the gene sequence interferes with the expression of the target plant gene. Reference is made to patent specification WO 97/20936 and European patent specification 0465572 for methods of implementing co-suppression approaches. The antisense, co-suppression or double stranded RNA molecules may also comprise a largely double-stranded RNA region, preferably comprising a nuclear localization signal, as described in WO 03/076619.

Any of these technologies for reducing gene expression can be used to coordinately reduce the activity of multiple genes. For example, one RNA molecule can be targeted against a family of related genes by targeting a region of the genes which is in common. Alternatively, unrelated genes may be targeted by including multiple regions in one RNA molecule, each region targeting a different gene. This can readily be done by fusing the multiple regions under the control of a single promoter.

A number of techniques are available for the introduction of nucleic acid molecules into a wheat cell, well known to workers in the art. The term "transformation" as used herein means alteration of the genotype of a cell, for example a bacterium or a plant, particularly a wheat plant, by the introduction of a foreign or exogenous nucleic acid. By "transformant" is meant an organism so altered. Introduction of DNA into a wheat plant by crossing parental plants or by mutagenesis per se is not included in transformation. As used herein the term "transgenic" refers to a genetically modified plant in which the endogenous genome is supplemented or modified by the random or site-directed integration, or stable maintenance in a replicable non-integrated form, of an introduced foreign or exogenous gene or sequence. By "transgene" is meant a foreign or exogenous gene or sequence that is introduced into a plant. The nucleic acid molecule may be replicated as an extrachromosomal element or is preferably stably integrated into the genome of the plant. By "genome" is meant the total inherited genetic complement of the cell, plant or plant part, and includes chromosomal DNA, plastid DNA, mitochondrial DNA and extrachromosomal DNA molecules. In an embodiment, a transgene is integrated in the wheat nuclear genome which in hexaploid wheat includes the A, B and D subgenomes, herein referred to as the A, B and D "genomes".

The most commonly used methods to produce fertile, transgenic wheat plants comprise two steps: the delivery of DNA into regenerable wheat cells and plant regeneration through in vitro tissue culture. Two methods are commonly used to deliver the DNA: T-DNA transfer using *Agrobacterium tumefaciens* or related bacteria and direct introduction

of DNA via particle bombardment, although other methods have been used to integrate DNA sequences into wheat or other cereals. It will be apparent to the skilled person that the particular choice of a transformation system to introduce a nucleic acid construct into plant cells is not essential to or a limitation of the invention, provided it achieves an acceptable level of nucleic acid transfer. Such techniques for wheat are well known in the art.

Transformed wheat plants can be produced by introducing a nucleic acid construct according to the invention into a recipient cell and growing a new plant that comprises and expresses a polynucleotide according to the invention. The process of growing a new plant from a transformed cell which is in cell culture is referred to herein as "regeneration". Regenerable wheat cells include cells of mature embryos, meristematic tissue such as the mesophyll cells of the leaf base, or preferably from the scutella of immature embryos, obtained 12-20 days post-anthesis, or callus derived from any of these. The most commonly used route to recover regenerated wheat plants is somatic embryogenesis using media such as MS-agar supplemented with an auxin such as 2,4-D and a low level of cytokinin, see Sparks and Jones, 2004).

Agrobacterium-mediated transformation of wheat may be performed by the methods of Cheng et al., 1997; Weir et al., 2001; Karma and Daggard, 2003 or Wu et al., 2003. Any *Agrobacterium* strain with sufficient virulence may be used, preferably strains having additional virulence gene functions such as LBA4404, AGL0 or AGL1 (Lazo et al, 1991) or versions of C58. Bacteria related to *Agrobacterium* may also be used. The DNA that is transferred (T-DNA) from the *Agrobacterium* to the recipient wheat cells is comprised in a genetic construct (chimeric plasmid) that contains one or two border regions of a T-DNA region of a wild-type Ti plasmid flanking the nucleic acid to be transferred. The genetic construct may contain two or more T-DNAs, for example where one T-DNA contains the gene of interest and a second T-DNA contains a selectable marker gene, providing for independent insertion of the two T-DNAs and possible segregation of the selectable marker gene away from the transgene of interest.

Any wheat type that is regenerable may be used; varieties Bob White, Fielder, Veery-5, Cadenza and Florida have been reported with success. Transformation events in one of these more readily regenerable varieties may be transferred to any other wheat cultivars including elite varieties by standard backcrossing. An alternative method using *Agrobacterium* makes use of an in vivo inoculation method followed by regeneration and selection of transformed plants using tissue culture and has proven to be efficient, see WO00/63398. Other methods involving the use of *Agrobacterium* include: co-cultivation of *Agrobacterium* with cultured isolated protoplasts; transformation of seeds, apices or meristems with *Agrobacterium*, or inoculation in planta such as the floral-dip method for *Arabidopsis* as described by Bechtold a al., 1993. This latter approach is based on the vacuum infiltration of a suspension of *Agrobacterium* cells. Alternatively, the chimeric construct may be introduced using root-inducing (Ri) plasmids of *Agrobacterium* as vectors.

Another method commonly used for introducing the nucleic acid construct into a plant cell is high velocity ballistic penetration by small particles (also known as particle bombardment or microprojectile bombardment) with the nucleic acid to be introduced contained either within the matrix of small beads or particles, or on the surface thereof as, for example described by Klein a al., 1987. This method has been adapted for wheat (Vasil, 1990). Microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* may be used (EP-A-486233). The genetic construct can also be introduced into plant cells by electroporation as, for example, described by Fromm et al., 1985 and Shimamoto et al., 1989. Alternatively, the nucleic acid construct can be introduced into a wheat cell such as a pollen cell by contacting the cell with the nucleic acid using mechanical or chemical means.

Preferred selectable marker genes for use in the transformation of wheat include the *Streptomyces hygroscopicus* bar gene or pat gene in conjunction with selection using the herbicide glufosinate ammonium, the hpt gene in conjunction with the antibiotic hygromycin, or the nptII gene with kanamycin or G418. Alternatively, positively selectable markers such as the manA gene encoding phosphomannose isomerase (PMI) with the sugar mannose-6-phosphate as sole C source may be used.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Methods and Materials

Carbohydrate Determination and Analysis.

Starch was isolated on small scale from both developing and mature wheat grain using the method of Regina et al., (2006). Large scale starch extraction was carried out following the method of Regina et al., (2004). Starch content was determined using the total starch analysis kit supplied by Megazyme (Bray, Co Wicklow, Republic of Ireland) and calculated on a weight basis as a percentage of the mature, unmilled grain weight. The starch content was then compared to control plants. Subtraction of the starch weight from the total grain weight to give a total non-starch content of the grain determined whether the reduction in total weight was due to a reduction in starch content.

The amylose content of starch samples was determined by the colorimetric (iodometric) method of Morrison and Laignelet (1983) with slight modifications as follows. Approximately 2 mg of starch was weighed accurately (accurate to 0.1 mg) into a 2 ml screw-capped tube fitted with a rubber washer in the lid. To remove lipid, 1 ml of 85% (v/v) methanol was mixed with the starch and the tube heated in a 65° C. water bath for 1 hour with occasional vortexing. After centrifugation at 13,000 g for 5 min, the supernatant was carefully removed and the extraction steps repeated. The starch was then dried at 65° C. for 1 hour and dissolved in urea-dimethyl sulphoxide solution (UDMSO; 9 volumes of dimethyl sulphoxide to 1 volume of 6 M urea), using 1 ml of UDMSO per 2 mg of starch (weighed as above). The mixture was immediately vortexed vigorously and incubated in a 95° C. water bath for 1 hour with intermittent vortexing for complete dissolution of the starch. An aliquot of the starch-UDMSO solution (50 μl) was treated with 20 μl of I_2-KI reagent that contained 2 mg iodine and 20 mg potassium iodide per ml of water. The mixture was made up to 1 ml with water. The absorbance of the mixture at 620 nm was measured by transferring 200 μl to microplate and reading the absorbance using an Emax Precision Microplate Reader (Molecular Devices, USA). Standard samples containing from 0 to 100% amylose and 100% to 0% amylopectin were made from potato amylose and corn (or potato) amylopectin (Sigma) and treated as for the test samples. The amylose content (percentage amylose) was determined from the absorbance values using a regression equation derived from the absorbances for the standard samples. Analysis of the amylose/amylopectin ratio of non-debranched starches may also be carried out

according to Case et al., (1998) or by an HPLC method using 90% DMSO for separating debranched starches as described by Batey and Curtin, (1996).

Statistical analysis of the amylose data was carried out using the 8th edition of Genstat for Windows (VSN International Ltd, Herts, UK).

The distribution of chain lengths in the starch was analysed by fluorophore assisted carbohydrate electrophoresis (FACE) using a capillary electrophoresis unit according to Morell et al., (1998) after debranching of the starch samples. The gelatinisation temperature profiles of starch samples were measured in a Pyris 1 differential scanning calorimeter (Perkin Elmer, Norwalk Conn., USA). The viscosity of starch solutions was measured on a Rapid-Visco-Analyser (RVA, Newport Scientific Pty Ltd, Warriewood, Sydney), for example using conditions as reported by Batey et al., (1997). The parameters measured included peak viscosity (the maximum hot paste viscosity), holding strength, final viscosity and pasting temperature. The swelling volume of flour or starch was determined according to the method of Konik-Rose et al., (2001). The uptake of water was measured by weighing the sample prior to and after mixing the flour or starch sample in water at defined temperatures and following collection of the gelatinized material.

Starch granule morphology was analysed by microscopy. Purified starch granule suspensions in water were examined under both normal and polarized light using a Leica-DMR compound microscope to determine the starch granule morphology. Scanning electron microscopy was carried out using a Joel JSM 35C instrument. Purified starches were sputter-coated with gold and scanned at 15 kV at room temperature.

β-Glucan levels were determined using the kit supplied by Megazyme (Bray, Co, Wicklow, Republic of Ireland).

Analysis of Protein Expression in Endosperm.

Specific expression of SBEI, SBEIIa and SBEIIb proteins in endosperm, in particular the level of expression or accumulation of these proteins, was analysed by Western blot procedures. Endosperm was dissected away from all maternal tissues and samples of approximately 0.2 mg were homogenized in 600 μl of 50 mM Kphosphate buffer (42 mM K_2HPO_4 and 8 mM KH_2PO_4), pH 7.5, containing 5 mM EDTA, 20% glycerol, 5 mM DTT and 1 mM Pefabloc. The ground samples were centrifuged for 10 min at 13,000 g and the supernatant aliquoted and frozen at −80° C. until use. For total protein estimation, a BSA standard curve was set up using 0, 20, 40, 60, 80 and 100 μl aliquots of 0.25 mg/ml BSA standard. The samples (3 μl) were made up to 100 μl with distilled water and 1 ml of Coomassie Plus Protein reagent was added to each. The absorbance was read after 5 min at 595 nm, using the zero BSA sample from the standard curve as the blank, and the protein levels in the samples determined. Samples containing 20 μg total protein from each endosperm were run on an 8% non denaturing polyacrylamide gel containing 0.34 M Tris-HCl (pH 8.8), acrylamide (8.0%), ammonium persulphate (0.06%) and TEMED (0.1%). Following electrophoresis, the proteins were transferred to a nitrocellulose membrane according to Morell et al., 1997 and immunoreacted with SBEIIa, SBEIIb or SBEI specific antibodies. Antiserum against wheat SBEIIa protein (anti-wBEIIa) was generated using a synthetic peptide having the amino acid sequence of the N-terminal sequence of mature wheat SBEIIa, AASPGKVLVPDGESDDL (SEQ ID NO: 16) (Rahman et al., 2001). Antiserum against wheat SBEIIb (anti-wHEIIb) was generated in an analogous manner using the N-terminal synthetic peptide, AGGPSGEVMI (SEQ ID NO: 17) (Regina et al., (2005). This peptide was thought to represent the N-terminal sequence of the mature SBEIIb peptide and furthermore was identical to the N-terminus of the barley SBEIIb protein (Sun et al., 1998). A polyclonal antibody against wheat SBEI was synthesised in an analogous manner using the N-terminal synthetic peptide VSAPRDYTMA-TAEDGV (SEQ ID NO: 18) (Morell et al., 1997). Such antisera were obtained from rabbits immunised with the synthetic peptides according to standard methods.

Enzyme Assay for SBE.

Enzyme activity assays of branching enzymes to detect the activity of all three isoforms, SBEI, SBEIIa and SBEIIb was based on the method of Nishi et al., 2001 with minor modification. After electrophoresis, the gel was washed twice in 50 mM HEPES, pH 7.0 containing 10% glycerol and incubated at room temperature in a reaction mixture consisting of 50 mM HEPES, pH 7.4, 50 mM glucose-1-phosphate, 2.5 mM AMP, 10% glycerol, 50 U phosphorylase a 1 mM DTT and 0.08% maltotriose for 16 h. The bands were visualised with a solution of 0.2% (WN) I_2 and 2% KI. The SBEI, SBEIIa and SBEIIb isoform specific activities were separated under these conditions of electrophoresis. This was confirmed by immunoblotting using anti-SBEI, anti-SBEIIa and anti-SBEIIb antibodies. Densitometric analysis of immunoblots using TotalLab software package (Nonlinear Dynamics Ltd, Newcastle, UK) which measures the intensity of each band was conducted to determine the level of enzyme activity of each isoform.

Starch branching enzyme (SBE) activity may be measured by enzyme assay, for example by the phosphorylase stimulation assay (Boyer and Preiss, 1978). This assay measures the stimulation by SBE of the incorporation of glucose 1-phosphate into methanol-insoluble polymer (α-D-glucan) by phosphorylase A. Activity of specific isoforms of SBE can be measured by this assay following purification of individual isoforms as described in Regina et al., 2004. The total soluble protein extracts were applied to a 3 ml β-cyclodextrin (β-CD) affinity column pre-equilibrated with the extraction buffer described above. The column was prepared by coupling β-CD to Epoxy-activated sepharose 6B (Amersham Biosciences, Uppsala, Sweden) following the manufacturer's instructions. The bound proteins (containing SBEs) were eluted using 1% β-CD in Phosphate buffer and then dialysed against buffer A (20 mM phosphate buffer, pH 8.0, 1 mM EDTA and 1 mM DTT). The dialysed samples were subjected to anion exchange chromatography using a 1 ml MonoQ column (Amersham Pharmacia), pre-equilibrated with buffer A. After elution of the unbound proteins, a 30 min linear gradient was applied by introducing buffer B (500 mM Phosphate buffer, pH 8.0, 1 mM EDTA, 1 mM DTT) into buffer A to elute the bound proteins.

SBE activity can also be measured by the iodine stain assay, which measures the decrease in the absorbency of a glucan-polyiodine complex resulting from branching of glucan polymers. SBE activity can also be assayed by the branch linkage assay which measures the generation of reducing ends from reduced amylose as substrate, following isoamylase digestion (Takeda et al., 1993a). Preferably, the activity is measured in the absence of SBEI activity. Isoforms of SBE show different substrate specificities, for example SBEI exhibits higher activity in branching amylose, while SBEIIa and SBEIIb show higher rates of branching with an amylopectin substrate. The isoforms may also be distinguished on the basis of the length of the glucan chain that is transferred. SBE protein may also be measured by using specific antibodies such as those described herein. Preferably, the SBEII activity is measured during grain development in the developing endosperm. SBEII protein levels are preferably mea-

sured in the mature grain where the protein is still present by immunological methods such as Western blot analysis.

DNA Analysis of Wheat Plants.

PCR analysis of transformed wheat plants or of plants to be tested for the presence of transgenes was performed on genomic DNA extracted from 1-2 cm^2 of fresh leaf material using the mini-prep method described by Stacey and Isaac, (1994). PCR assays to determine the presence of the hairpin RNA constructs used the primers SBEIIa-For: 5'-CCCGCTGCTTTCGCTCATTTTG-3 (SEQ ID NO: 19) and SBEIIa-Rev: 5'-GACTACCGGAGCTCCCACCTTC-3' (SEQ ID NO: 20) designed to amplify a fragment (462 bp) from the SBEIIa gene. Reaction conditions were as follows: "hot start" (94° C., 3 min) followed by 30 cycles of denaturation (95° C., 30 sec), annealing (55° C., 30 sec), extension (73° C., 2 min) followed by 1 cycle at 73° C. (5 min). Reaction products were analysed by agarose or polyacrylamide gel electrophoresis.

Southern blot hybridization analysis was performed on DNA from a larger scale (9 ml) extraction from lyophilized ground tissue (Stacey and Isaac, 1994). DNA samples were adjusted to 0.2 mg/ml and digested with restriction enzymes such as HindIII, EcoRI and KpnI. Restriction enzyme digestion, gel electrophoresis and vacuum blotting are carried out as described by Stacey and Isaac, (1994). Digoxygenin-labelled probes including the intron 3 region of the ds-SBEII constructs are produced by PCR according to the method of McCreery and Helentjaris, (1994). Hybridization of the probes to the Southern blot and detection by chemiluminescence are performed according to the method of McCreery and Helentjaris, (1994).

Transformation of Wheat by *Agrobactaerium.*

Genetic constructs for transformation of wheat were introduced by electroporation into the disarmed *Agrobacterium tumefaciens* strain LBA4404 carrying the vir plasmid pAL4404 and pSB1, with subsequent selection on media with spectinomycin. Transformed *Agrobacterium* strains were incubated on solidified YEP media at 27° C. for 2 days. Bacteria were then collected and re-suspended in TSIM1 (MS media with 100 mg/l myo-inositol, 10 g/l glucose, 50 mg/l MES buffer pH5.5) containing 400 mM acetosyringone to an optical density of 2.4 at 650 nm for wheat inoculation.

Wheat plants (variety NB1, a Spring wheat variety obtained from Nickerson Seeds Ltd, Rothwell, Lincs.) were grown in a glasshouse at 22/15° C. day/night temperature with supplemented light to give a 16 hour day. Tillers were harvested approximately 14 days post-anthesis (embryos approximately 1 mm in length) to include 50 cm tiller stem. All leaves were then removed from the tillers except the flag leaf, which was cleaned to remove contaminating fungal spores. The glumes of each spikelet and the lemma from the first two florets were then carefully removed to expose the immature seed. Generally, only these two seed in each spikelet were uncovered. This procedure was carried out along the entire length of the inflorescence. The ears were then sprayed with 70% IMS as a brief surface sterilization.

Agrobacterium suspensions (1 μl) were inoculated using a 10 μl Hamilton syringe into the immature seed approximately at the position of the scutellum:endosperm interface so that all exposed seed were inoculated. The tillers were then placed in water, covered with a translucent plastic bag to prevent seed dehydration, and placed in a lit incubator for 3 days at 23° C., 16 hr day, 45 $\mu Em^{-2}s^{-1}$PAR. After 3 days of co-cultivation, the inoculated immature seed were removed and surface sterilized with 70% ethanol (30 sec), then 20% bleach (Domestos, 20 min), followed by thorough washing in sterile distilled water. Immature embryos were aseptically isolated and placed on W3 media (MS supplemented with 20 g/l sucrose and 2 mg/l 2,4-D and solidified with 6 g/l Type I agarose, Sigma) with the addition of 150 mg/l Timentin (W3T medium) and with the scutellum uppermost (20 embryos per plate). Cultures were placed at 25° C. in the light (16 hour day, 80 $\mu Em^{-2}s^{-1}$PAR). The development of the embryonic axis on the embryos was assessed about 5 days after isolation and the axis was removed where necessary to improve callus production. The embryos were maintained on W3T for 4 weeks, with a transfer to fresh media at 2 weeks post-isolation and assessed for embryogenic capacity.

After 4 weeks growth, callus derived from the inoculated embryos was very similar to control callus obtained from uninoculated embryos plated on W3T medium. Presence of the bacteria did not appear to have substantially reduced the embryogenic capacity of the callus derived from the inoculated embryos. Embryogenic calli were transferred to W3 media with 2 mg/l Asulam or geneticin at 25 mg/l and 150 mg/l Timentin (W32AT medium). Calli were maintained on this media for a further 2 weeks and then each callus was divided into 2 mm-sized pieces and re-plated onto W32AT. Control embryos derived from inoculations with the LBA4404 without binary vector constructs did not produce transformed callus on selection media.

After a further 2 weeks culture, all tissue was assessed for development of embryogenic callus: any callus showing signs of continued development after 4 weeks on selection was transferred to regeneration media (RMT-MS with 40 g/l maltose and 150 mg/l Timentin, pH 5.8, solidified with 6 g/l agarose, Sigma type 1). Shoots were regenerated within 4 weeks on this media and then transferred to MS30 with 150 mg/l Timentin for shoot elongation and rooting. Juvenile plants were then transferred to soil mixture and kept on a misting bench for two weeks and finally transferred to a glasshouse.

Alternative *Agrobacterium* strains such as strain AGL1 or selectable markers such as genes encoding hygromycin resistance can also be used in the method.

EXAMPLE 2

Inhibition of SBEIIa Genes in Wheat Using Four Hairpin RNA Constructs

Four hairpin RNA (dsRNA) constructs were made to reduce the expression of i) the SBEIIa, or ii) the SBEIIa, SBEIIb and SBEI genes of wheat. In each construct, the DNA encoding the hairpin RNA was linked to a high molecular weight glutenin (HMWG) promoter sequence obtained from a wheat Dx5 gene to provide endosperm-specific expression of the hairpin RNA, and a transcription terminator sequence from the nopaline synthase gene from *Agrobacterium* (nos3'). This promoter provided for endosperm-specific expression of the synthetic genes encoding the hairpin RNAs.

hp5'-SBEIIa.

The construction and use of the first of the constructs, designated as hp5'-SBEIIa, is described in Regina et al., (2006). The hp5'-SBEIIa construct contained 1536 bp of nucleotide sequence amplified by PCR from the wheat SBEIIa gene (GenBank Accession number AF338431). This included a 468 bp sequence that comprises the whole of exons 1 and 2 and part of exon 3 (nucleotide positions 1058 to 1336, 1664 to 1761 and 2038 to 2219 (that includes nucleotide positions 1 to 578 of *Aegilops tauschii* cDNA encoding SBEIIa, GenBank accession number AF338431.1) with EcoRI and KpnI restriction sites on either side (fragment 1), a 512 bp sequence consisting of part of exons 3 and 4 and the whole of intron 3 of SBEIIa (nucleotide positions 2220 to

2731) with KpnI and SacI sites on either side (fragment 2) and a 528 bp fragment consisting of the complete exons 1, 2 and 3 of SBEIIa (nucleotide positions 1058 to 1336, 1664 to 1761 and 2038 to 2279 in AF338431, that includes nucleotide positions 1 to 638 of *Aegilops tauschii* SBEIIa cDNA, GenBank accession number AF338431.1) with BamHI and Sad sites on either side (fragment 3). Fragments 1, 2 and 3 were then ligated so that the sequence of fragment 3 was ligated to fragment 2 in the antisense orientation relative to fragment 1. The hairpin RNA constructs were initially generated in the vector pDVO3000 which contains the HMWG promoter sequence and nos3' terminator.

hpc-SBEIIa.

The SBEIIa construct designated hpc-SBEIIa comprised a 293 basepair DNA fragment corresponding to nucleotides 1255 to 1547 of the SBEIIa cDNA (GenBank Accession No. AF338432.1), which corresponds to part of exon 12, exons 13 and 14 and part of exon 15 of the SBEIIa gene. This region of SBEIIa was chosen because it had only about 81% identity to the nucleotide sequence of the corresponding region of SBEIIb cDNA, thus increasing the chance of specificity of silencing of SBEIIa but not SBEIIb.

hp3-SBEIIa.

The SBEIIa construct designated hp3'-SBEIIa comprised a 130 basepair DNA fragment corresponding to nucleotides 2305 to 2434 of the SBEIIa cDNA, corresponding to part of exon 21, exon 22 and part of the 3' untranslated region (3' UTR) of the SBEIIa gene.

hp-Combo.

The hairpin RNA construct designated hp-combo comprised regions of the wheat SBEI gene in addition to parts of the SBEIIa gene, and contained i) a 417 basepair sequence corresponding to nucleotides 1756 to 2172 from the SBEIIa cDNA, corresponding to part of exon 16, exons 17 to 19, and part of exon 20, and ii) a 357 basepair sequence corresponding to nucleotides 267 to 623 of an SBEI cDNA (GenBank Accession No. AF076679), corresponding to part of exon 3, exon 4, and part of exon 5 of the SBEI gene. The SBEIIa gene fragment had about 86% identity to the corresponding region of the SBEIIb gene, including several regions of 23 consecutive nucleotides with 100% identity to their corresponding regions of SBEIIb, and therefore the combination construct was designed with the expectation that it would reduce expression of the genes encoding SBEIIb as well as the genes encoding SBEIIa and SBEI in wheat.

Two copies of each of the fragments described above were inserted, one in sense and the other in antisense orientation, into a suitable vector, such that a rice tubulin gene intron was present between the two copies. The synthetic gene was inserted into a binary vector and used to transform wheat.

These constructs were used to transform wheat as described in Example 1. The numbers of independent wheat transgenic lines that were PCR positive for the respective constructs were as follows: hp5'-SBEIIa, 27; hpc-SBEIIa, 10; hp3'-SBEIIa, 10; and hp-combo, 63.

Analyses of Transgenic Plants: DNA Analysis.

PCR analysis was performed to detect one or more of the transgenes in the regenerated plants using genomic DNA extracted from 1-2 cm^2 of fresh leaf material using the miniprep method described by Stacey and Isaac, (1994). PCR reactions were performed for plants transformed with the hp5'-SBEIIa transgene, for example, using the primers SBEIIa-For: 5'-CCCGCTGCTTTCGCTCATTTTG-3' (SEQ ID NO: 19) and SBEIIa-Rev: 5'-GACTACCGGAGCTCCCACCTTC-3' (SEQ ID NO: 20). These PCR reactions were designed to amplify a fragment of about 462 bp from the SBEIIa gene. Reaction conditions were as follows: "hot start" (94° C., 3 min) followed by 30 cycles of denaturation (95° C., 30 sec), annealing (55° C., 30 see) and extension (73° C., 2 min), followed by 1 cycle at 73° C. (5 min).

Starch Granule Morphology.

The morphology of starch granules from mature T1 seed obtained from the T0 transformed wheat plants was observed by light microscopy. Ten individual grains from each of 25 T0 hp5'-SBEIIa plants were analysed. Each endosperm was gently crushed to release the starch granules which were dispersed in water and visualized under a light microscope. Of the 25 lines analysed, 12 had grains with distorted granules although the visual observation revealed varying levels of distortion in different seeds. Nine seeds from each of the plants transformed with the hpc-SBEIIa, hp3'-SBEIIa and hp-combo transgenes were similarly analysed for morphological alterations in the starch granules. In this case, half-seeds were analysed so that each remaining halfseed could be grown into a T1 plant, thus preserving each line. Fifty-five out of 63 hp-combo lines had seeds with altered granule morphology with varying levels of distortion. All of the ten hp5'-SBEIIa lines had seeds with altered starch granule morphology, again with varying levels of distortion. No significant starch granule morphology alteration was observed in any of the SBEIIa 3' lines. Distorted starch granules are an indicator of elevated amylose levels in the starch of the endosperm, typically above 50% amylose, or above 70% amylose for highly distorted starch granules. This indicated that a range in the extent of the phenotype was observed for each of the effective silencing constructs.

Protein Expression by Western Blotting in Developing Endosperm.

Four to seven T2 developing endosperms from T1 transgenic lines were analysed for the level of SBEIIa and SBEIIb proteins by Western blotting using anti-SBEIIa and anti-SBEIIb antibodies, respectively. In the case of hp-combo lines, SBEI expression was also analysed using anti-SBEI antibody. Total SBEII protein levels (SBEIIa and SBEIIb) from selected transgenic lines were calculated as a percentage of the level in the wild-type (variety NB1) and is shown in Table 11. Amylose levels in mature grain from the transgenic lines, calculated as a percentage of the total starch in the grain, was also determined (Table 11) using an iodometric method as described in Example 1. This is represented graphically in FIG. **5**.

A range of expression levels of SBEIIa and SBEIIb were obtained in the grain of the transgenic plants of independent lines. Such a range is normally expected in transgenic lines obtained with any one construct, due to the variation in integration sites of the transgene in different transgenic events, commonly referred to as "position effect". The range of expression levels seen in these experiments was extended because it was observed that the four constructs were not equally efficient in reducing the expression of the SBEIIa and SBEIIb genes. In particular, the extent of reduction in the expression of SBEIIb caused by the hp-combo construct in some transformed lines did not correlate with the extent of reduction in expression of SBEIIa, for example lines 679.5.3 and 672.2.3. However, all of the constructs reduced expression of the corresponding genes in a majority of transformed lines.

When the percentage of amylose was plotted against the total SBEII protein level and a curve of best fit generated from the data points (see FIG. **5**), it was observed that reducing the total SBEII by at least 75% relative to the wild-type yielded an amylose content of 50% (w/w) or greater in the endosperm

starch. Reducing the total SBEII activity by at least 40% relative to the wild-type yielded an amylose content of at least 40% (w/w).

When the percentage of amylose was plotted against the remaining SBEIIa protein level, a very similar curve was obtained (see FIG. **6**), leading to the conclusion that the level of SBEIIa in wheat endosperm was the primary determinant of the amylose level in the starch, and that the levels of SBEIIb and SBEI were secondary determinants.

The amylose model was further developed based on three sets of inputs (FIG. **6**):

(1) theoretical data based on relative expression levels of SBEIIa and SBEIIb and amylose data from transgenics

(2) amylose data for single and double nulls and theoretical data based on relative expression levels of SBEIIa and SBEIIb

(3) measured amylose data and measured SBEIIa and SBEIIb levels from the "additional construct" transgenics

In FIG. **6**, a power curve has been fitted to this data. Bringing together these three data sets generated a model that was highly consistent between input types, reinforcing the model as a predictive tool. The model predicted the importance of generating multiple mutations in SBEII genes in order to generate high amylose in bread wheat or tetraploid wheat.

EXAMPLE 3

Cloning and Comparison of SBEII Gene Sequences from Wheat

Isolation of SBEII genes from an *Aegilops tauschii* genomic library and their characterisation by PCR are described in WO99/14314 and WO200162934-A. DNA sequences from the intron 5 region of SBEIIa gene of the A, B and D genomes are described in WO200162934-A. Further research has led to obtaining sequences from other regions of wheat SBEIIa genes from different wheat genotypes and further characterisation of the homoeologous genes, for example as follows. The exons 12 to 14 region of SBEIIa was amplified from the hexaploid wheat variety Chara using the primers AR2aE12F07 (5'-CATTCGTCAAATAATACCCTTGACGG-3' (SEQ ID NO: 21)) and AR2 aE14R07 (5'-CTTCACCAATGGATACAGCATCAG-3 (SEQ ID NO: 22)). This yielded a PCR product of about 656 bp which was presumed to be a mixture of the amplified fragments from each of the three homoeologous genes. This product was sequenced following cloning in a TOPO vector. Three polymorphic sequences were obtained that covered the region between exon 12 to 14 (FIG. **7**). Based on PCR analysis of Chinese Spring chromosome engineered lines using cleavage amplified polymorphic (CAP) markers, the sequence F1-1 was assigned to the D genome, the sequence F1-13 was assigned to the B genome and the sequence F1-15 was assigned to the A genome as detailed in Example 4.

The intron 3 region of SBEIIa was amplified from two hexaploid wheat varieties, Sunco and Tasman, using the primer pair AR2akpnIF (5'-GGTACCGGCAAATATACGAG ATTGACCCG-3' (SEQ ID NO: 23)) and AR2aSacIR (5'-GAGCTCCCACCTTCATGTT GGTCAATAGC-3' (SEQ ID NO: 24)). Three polymorphic sequences were obtained from each of Sunco and Tasman (FIG. **8**). By comparison with the wheat SBEIIa D genome sequence (GenBank Accession No. AF338431.1), the sequences Tasman 0257 and Sunco 0242 were assigned to the D genome. Tasman 0272 and Sunco 0241 sequences were assigned to the B genome based on mapping a polymorphic marker based on a single nucleotide polymorphism in a segregating population. The sequences Tasman 0264 and Sunco 0243 appeared to be different from the B and D genome sequences and it was concluded they must be from the A genome. Genotype specific polymorphisms were also observed for this region of SBEIIa between Sunco and Tasman in each of the three genomes.

The exon 3 region of SBEIIa from Chinese Spring (CS) was amplified using the primers AR2aexon3F (5'-GATACCTGAAGATATCGAGGAGC-3' (SEQ ID NO: 25)) and AR2aexon3R (5'-CGGTAGTCAAGATGGCTCCG-3' (SEQ ID NO: 26)). Three polymorphic sequences were obtained (FIG. **9**). Comparison with the wheat SBEIIa gene (GenBank Accession No. AF338431.1) revealed that the sequence CS exon 3a was from the D genome. The sequence CS exon 3b was found to be from the B genome based on the 100% identity with the GenBank Accession No. FM865435 which was reported to be from a bread wheat 2B chromosome. The third sequence CS exon 3d showed 99% identity with the GenBank Accession No. Y11282.1, which in turn had a high degree of identity (99%) with a partial coding sequence reported from the A genome of Chinese Spring (GenBank Accession No. EU670724). This led to the prediction that the sequence CS exon 3d was from the A genome.

The exon 1 region of SBEIIa from CS was amplified using the primers AR2aexon1F (5'-CACACGTTGCTCCCCCTTCTC-3' (SEQ ID NO: 29)) and AR2aexon1R (5'-GAGAGGAGTCCTTCTTCCTGAGG-3' (SEQ ID NO: 28)). The sequences were obtained (FIG. **10**). Alignment with SBEII GenBank accessions led to assigning the sequence CS exon 1a to the B genome (100% homology to FM865435), CS exon 1b to the A genome (99% homology to Y11282.1) and CS exon 1c to the D genome (100% homology to AF338431.1).

SBEIIa gene sequences were also obtained from the diploid progenitors or relatives of breadwheat, *Triticum urartu* which is thought to be the A genome progenitor of breadwheat, *Aegilops speltoides* (also known as *Triticum speltoides*) which is thought to be the B genome progenitor, and *Aegilops tauschii* which is thought to be related closely to the D genome progenitor. Gene fragments were obtained from these species as follows: Ten primers were designed based on the nucleotide sequence of the SBEIIa gene of the D genome (Accession No. AF338432) or its complement and covering the whole of that sequence. These primer sets were used to amplify fragments of the SBEIIa genes of diploid species by PCR. Using the 10 primers, 16 combinations were used in PCBs with DNA from the diploid species *T. urartu* (AA genome), *A. speltoides* (BB), *A. tauschii* (DD) and the tetraploid species *T. durum* (AABB genome). In total, 35 fragments were selected from these amplifications which were of sufficient quality for sequencing, to determine their nucleotide sequences. The sequences will be compared and edited using Contig Express and combined sequences determined for the progenitor SBEIIa genes from the diploids. Polymorphisms such as SNPs or insertions/deletions will be identified which can be used to distinguish the genes on the A, B and D genomes, and specific primers designed using Amplifier for identification of mutants.

The nucleotide sequence of the exon 11-22 region of the SBEIIa gene from *T. urartu* is shown in SEQ ID NO: 13, of the exons 3-8 as SEQ ID NO: 15 and of exons 1-3 as SEQ ID NO: 14. The nucleotide sequence of the entire SBEIIa gene of *A. tauschii* is provided in WO2005001098 (incorporated herein by reference).

Mapping of SBEIIa and SBEIIb-Genetic Linkage of SBEIIa and SBEIIb in Wheat.

The SBEIIa and SBEIIb genes were both located on the long arm of wheat chromosome 2 (Regina et al, 2005; Rah-

man et al., 2001) and based on these reports were thought to be linked, although it was not known exactly how close the linkage was. Genetic mapping of the SBEIIa and SBEIIb genes was carried out using a segregating population obtained from a 4-way cross involving the parental cultivars Baxter, Yitpi, Chara and Westonia. The analysis of the population for recombinants between the genes revealed only one recombinant out of approximately 900 progeny. From this data, it was calculated that the genetic distance between SBEIIa and SBEIIb was only 0.5cM, which was a very tight linkage between the two genes.

To determine the physical distance between the two genes, a BAC library of *Aegilops tauschii* constructed by Moullet et al., (1999) was screened to identifying SBEII containing clones. Hybridisation probes labelled with ^{32}P were prepared from the 5' and 3' regions from each of the SBEIIa and SBEIIb genes and used to screen the BAC library. When screened with a mixture of the four probes, nine clones were identified with positive hybridisation signals. The nine clones were then screened separately with each of the probes and three clones selected. One of them (BAC2) was fully sequenced and shown to contain a full length SBEIIb gene. Of the other clones, BAC1 was shown to contain a SBEIIa gene by partial direct sequencing and BAC3 appeared to contain portions of both of the SBEIIa and SBEIIb genes as shown by PCR. This indicated how closely the two genes are physically linked. BAC1 and BAC3 will be fully sequenced. This physical data confirmed the close genetic linkage.

It was therefore predicted that deletion mutations created by agents such as radiation which affected one of the genes were likely to extend into or across both genes i.e. be null for both genes. Furthermore, this suggested to us the possibility that such deletion mutants might be viable and have wild-type fitness. At least, the observed tight linkage raised the possibility of obtaining mutants with relatively small deletions which did not extend to other linked genes needed for viability or fitness. Such mutants were therefore sought as described below in Examples 5-7.

EXAMPLE 4

Distinguishing the SBEIIa and SBEIIb Homoeologous Genes in Wheat

Based on the sequence polymorphisms obtained in Example 2, PCR assays were designed and prepared to distinguish the homoeologous SBEIIa genes in breadwheat. A nested primer pair, AR2aI13genomeF2 (5'-GTACAATTTTACCTGATGAGATC ATGG-3' (SEQ ID NO: 29)) and AR2aI13genomeR2 (5'-CTTCAGGAATGGATACAGCATCAG-3' (SEQ ID NO: 30)) was designed to amplify a 207 bp product from the region between the exons 12 to 14 of wheat SBEIIa. When digested with two restriction enzymes, Ssp1 and Mse1, the product amplified using these primers from Chinese Spring (CS) yielded four clear bands of sizes 207 bp, 147 bp, 99 bp and 108 bp. Use of this PCR marker assay on CS chromosome engineered lines revealed that the 207 bp product came from the A genome, the 147 bp product came from the B genome and the 99 bp and 108 bp products came from the D genome (FIG. **11**).

Based on SBEIIa sequences from the diploid ancestors of wheat namely *Triticum urartu* for genome A, *Aegilops speltoides* for genome B and *Aegilops tauschii* for genome D, primer pairs were designed that could specifically amplify fragments from different regions of the SBEIIa genes from the different genomes and distinguish them (Tables 4 to 8). Tables 6 to 8 list some of the nucleotide polymorphisms (column labelled SNP) and the sizes of the amplified fragments obtained when the designated primer pairs are used. These same primer combinations can be used to distinguish the A and B genome homoeologous SBEIIa genes from durum wheat.

Development of some PCR primer sets distinguishing the homoeologous SBEIIb genes from the A, B and D genomes of breadwheat and the identification of SBEIIb in each of these genomes in hexaploid wheat are described in WO200162934-A. Based on SBEIIb sequences from the diploid ancestors of wheat namely *Triticum urartu* for genome A, *Aegilops speltoides* for genome B and *Aegilops tauschii* for genome D, primer pairs that could amplify specifically each of the three genomes from different regions of SBEIIb were designed (Tables 9 to 10). These same primer combinations can be used to distinguish the A and B genome homoeologous SBEIIb genes from durum wheat.

EXAMPLE 5

Generation and Identification of SBEII Mutants

Mutagenesis of Wheat by Heavy Ion Bombardment.

A mutagenised wheat population was generated in the wheat variety Chara, a commonly used commercial variety, by heavy ion bombardment (MB) of wheat seeds. Two sources of heavy ions were used namely carbon and neon, for mutagenesis which was conducted at Riken Nishina Centre, Wako, Saitama, Japan. Mutagenised seeds were sown in the greenhouse to obtain the M1 plants. These were selfed to produce the M2 generation. DNA samples isolated from each of approximately 15,000 M2 plants were individually screened for mutations in each of the SBEIIa and SBEIIb genes using the genome specific PCR primers for SBEIIa ARIIaF2/ARIIaR2) and SBEIIb (ARA19F/ARA23R) (diagnostic PCR). Each of the PCR reactions on wild-type DNA samples yielded 3 distinct amplification products which corresponded to the amplified regions of SBEIIa or SBEIIb genes on the A, B and D genomes, whereas the absence of one of the fragments in the PCRs from mutagenised M2 samples indicated the absence of the corresponding region in one of the genomes, i.e. the presence of a mutant allele in which at least part of the gene was deleted. Such mutant alleles would almost certainly be null alleles.

Screening of the M2 lines using the genome specific primer pairs identified a total of 34 mutants which were mutant for the SBEIIa and/or SBEIIb genes. The mutants in SBEIIa were then screened for the presence of the SBEIIb genes, and vice versa. The identified mutants were thereby classified into three groups: "Type I" where both SBEIIa and SBEIIb genes were mutant i.e. lacking both wild-type genes in one genome, "Type 2", where only the SBEIIa gene was mutant while the SBEIIb gene was wild-type, and "Type 3", where only the SBEIIb gene was mutant and the SBEIIa gene was wild-type in the particular genome. Since the SBEIIa genes on the A, B and D genomes were distinguished by the diagnostic PCR reactions, and likewise the SBEIIb genes, the mutant alleles could be assigned to one of the genomes according to which amplification product was absent. As used herein, the designation "A1" refers to the genotype where both the SBEIIa and SBEIIb genes on the A genome were mutant, "A2" refers to the genotype where the SBEIIa gene was mutant and the SBEIIb gene on the A genome was wild-type, and "A3" refers to the genotype where the SBEIIa gene was wild-type and the SBEIIb gene on the A genome was mutant. The designations "B1", "B2", "B3", "D1", "D2" and "D3" have the analogous

meanings for the B and D genomes. Mutants of each of these nine possible types were identified among the collection of 34 mutants.

The extent of the chromosome deletion in each of the 34 mutants was determined by microsatellite mapping. Microsatellite markers previously mapped to the long arm of chromosomes 2A, 2B and 2D (Table 12) were tested on these mutants to determine the presence or absence of each marker in each mutant. Mutant plants in which either all or most of the specific chromosome microsatellite markers were retained, based on the production of the appropriate amplification product in the reactions, were inferred to be relatively small deletion mutants. Such mutants were preferred, considering that it was less likely that other, important genes were affected by the mutations. The identified mutants and the results from the microsatellite mapping are summarized in Table 13.

Crossing of Mutants.

Mutant plants that were homozygous for smaller deletions as judged by the microsatellite marker analysis were selected for crossing to generate progeny plants and grain which had mutant SBEII alleles on multiple genomes. F1 progeny plants from the crosses were selfed, and F2 seed obtained and analysed for their SBEII genotype. Screening 12 such F2 populations led to the identification of 11 different combinations of mutant alleles ("double nulls") (Table 14). The double null combination of the B1D1 genotype was not obtained in the twelfth cross in spite of screening more than 1200 F2 progeny of that particular cross. One possible explanation for this might be the presence of a critical gene in the vicinity of the SBEII locus in the B and D genomes, but not in the A genome, and hence the combination of the B1 and D1 double null mutations might render the seed non viable. Twenty seven combinations of double-null mutants are theoretically possible, and more F2 populations will be screened to identify the other combinations.

EXAMPLE 6

Amylose Content of Single and Double Null SBEII Mutants of Wheat

The percentage of amylose in the grain starch of single and double null plants described in Example 5 was determined using the iodometric method as described in Example 1. A scatter diagram plotting amylose content (Y-axis) against the mutant line number (X-axis) is shown in FIG. **4**. The amylose content in the mutant grains ranged from 27.3 to 38.7%. The amylose content of wild-type (unmutagenised) Chara samples ranged from 27.4% to 29.5%. Twenty six lines recorded an amylose content of above 34%. It was observed that of these 26 lines, 20 were double nulls, of which some were replicates from the same cross, of either Type 1 or Type 2 combinations. In other words, there was a trend in significantly increasing amylose content in Type 1 and Type 2 double null combinations compared to the amylose content in single null grains.

Importantly, and unexpectedly prior to this study, none of the double null mutant grains had starch with greater than 40% amylose. This included the A1B1, A1D1 and B1D1 genotypes which each contained four SBEIIa and four SBEIIb null alleles and retained two wild-type SBEIIa and two wild-type SBEIIb alleles. This observation was consistent, however, with the prediction made from the data in Example 2. It was therefore concluded that to obtain wheat grain with more than 40% amylose by combining mutations, the grain needed to have more than four mutant alleles of SBEIIa, or alternatively, if only four mutant alleles of SBEIIa were present, more than four mutant SBEIIb alleles in combination with the four SBEIIa alleles, preferably all six SBEIIb genes being mutant. It was also suggested from the data that the SBEIIa genes on each of the A, B and D genomes were expressed at similar levels relative to each other, i.e. SBEIIa expression in breadwheat was not predominantly from any one genome.

It was interesting to note that the "A3" and "A3D3" genotypes had low amylose contents consistent with the data in Example 2, confirming that SBEIIb had a lesser role in determining amylose content in wheat relative to SBEIIa.

EXAMPLE 7

Crosses in Attempts to Create Triple Null Mutants

In order to create mutant lines with more than four SBEIIa mutant alleles, some of the single null and double null lines were crossed and the F2 progeny of these crosses analysed using the diagnostic PCR assays. The assays tested for the presence of the three SBEIIa and three SBEIIb genes and were therefore used in an attempt to identify plants which had null mutations in the SBEIIa and/or SBEIIb genes in each of the A, B and D genomes (triple null lines for SBEIIa and/or SBEIIb). The crosses that were carried out in a first experiment and the genotypes of the parental lines and potential triple null F2 progeny are listed in Table 15.

Starch granule morphology was analysed by microscopy of selected normal looking and shriveled/shrunken F2 seeds from these crosses. Six shrivelled/shrunken seeds were selected, 5 from the 08/dd cross and 1 from the 08/bb cross, each of which were obtained from crosses between a D2 single null parent plant and an A1B2 double null parent plant. Each of the six seeds showed severe distortion of starch granules, showing abnormal, distorted shapes for most granules in the seeds which was similar to granules observed in transgenic seeds with elevated amylose levels (Example 2). Inspection of a number of shrivelled/shrunken seeds and selected odd looking seeds from the other crosses revealed no altered starch granule morphology, indicating that the phenotype observed in 08/dd and 08/bb seeds was genotype specific and not due to developmental problems during seed development.

Starch isolated from 6 of the seeds having distorted starch granules was pooled and tested for amylose content using the iodometric method as described in Example 1. The amylose content of the pooled sample was measured to be 67% (Table 16). Amylose levels in the wild-type seeds (control) of cultivars Cadoux and Chara were approximately 35%.

Genotypic Analysis of Seeds with Altered Starch Granule Morphology.

The seeds from the crosses 08/dd and 08/bb with altered starch granule morphology were sown and the resultant plants grown in the greenhouse. DNA extracted from the plants was analysed using the genome specific primers for SBEIIa and SBEIIb described in Example 3. Results from the PCR assays indicated that each of these seeds were homozygous double null mutants with an A1B2, B2D2 or A1D2 genotype while the third (wildtype) gene was present in either the homozygous or heterozygous state. DNA from these plants were further tested using quantitative PCR (Real-time PCR, Rotorgene 6000) using genome specific individual primer pairs to assay the presence or absence and the homozygosity or heterozygosity of the 3 SBEIIa genes in the plants. The primer pairs used for SBEIIa were Snp6for/Arev5 (SEQ ID NO: 51/SEQ ID NO: 61) (A genome, 205 bp amplification prod-

uct), BSnp4/Arev5 (SEQ ID NO: 55/SEQ ID NO: 61) (B genome, 494 bp amplification product) and DSnp7for/Drev1 (SEQ ID NO: 58/SEQ ID NO: 62) (D genome, 278 bp amplification product). In order to normalize the SBEIIa amplification reactions, a primer pair (SJ156/SJ242) which amplified a 190 bp product from the CslF6 gene, which is a cell-wall biosynthesis gene expected to be equally expressed in all of the plants and located on wheat Chromosome 7, was used in control amplifications. DNA from a wild-type plant from the mutagenised population, designated 2B2, and from wild-type cv. Chinese Spring (CS) were used as control templates. The relative concentration values generated in the reactions with the SBEIIa primers were normalised with the value for Cslf6 primers for each template DNA preparation. The values for the potential triple null plants and CS were calculated relative to line 2B2.

Out of these three primer pairs, the D genome primers produced a clear single band for one plant designated as S14 which enabled quantitation. No bands were obtained for the SBEIIa genes on the A and B genomes of S14, indicating it was homozygous for the mutant alleles on these genomes. The quantitation indicated that S14 had approximately 30-50% of the D allele complement compared to 2B2 whereas CS gave a value of approximately 95% of 2B2 for the D genome SBEIIa gene. This showed that S14 which gave seed with amylose levels of about 67% was homozygous for SBEIIa null mutations for two of the genomes (A and B) and heterozygous for the third genome (D), in addition to being homozygous for SBEIIb null mutation in the A genome. That is, S14 had an A1 (homozygous), B2 (homozygous), D2/+ (heterozygous) genotype. In a similar fashion, the quantitative PCR showed that plant designated as S24 had a B2 (homozygous), D2 (homozygous) and A1 (heterozygous) genotype, The PCR analysis showed that the remaining 5 plants had the following genotypes: 08dd9-B4 was homozygous for an A1132 genotype i.e. homozygous mutant for SBEIIa and SBEIIb on the A genome, homozygous mutant SBEIIa and wild-type SBEIIb on the B genome and homozygous wildtype for both genes on the D genome, while 08bb11-D9 was homozygous for a B2D2 genotype and S28 and S22 were homozygous for an A1D2 genotype

Analysis of F3 Seeds.

Seeds of the S28, S22, S14 and S24 lines were sown in the greenhouse, the resultant plants were selfed, and seeds (F3 generation) obtained from each plant. It was observed that the fertility of the plants was affected, in that the number of seeds per head and the percentage of spikes which were fertile were significantly reduced compared to wild type, single null and other double null mutants grown at the same time and under the same conditions, but not abolished (Table 17).

Starch granule morphology was determined by light microscopy on 100-200 seeds from each of the lines S28, S14 and S22. From the line S22, 102 F3 seeds were identified with distorted starch granules from among 200 seeds tested. The data revealed a distortion of the segregation ratios away from the expected 1:2:1 (homozygous mutant: heterozygote: wild-type) with a higher number of normal phenotypes than expected. In order to see whether a homozygous plant with a high amylose phenotype could be identified, 102 seeds with distorted granules were placed in conditions suitable for germination. Sixty one out of the 102 seeds germinated. DNA from these 61 plants were analysed by SBEIIa genome specific PCR and all 61 plants appeared to be double null of an A1D2 genotype, with no homozygous triple nulls identified. The wild-type SBEIIa gene on the B genome was shown to be heterozygous i.e. both wildtype and mutant alleles were present for the 13 genome.

The 41 seeds which had distorted starch granules but had not germinated were analysed for their SBEIIa genotype. Many of these were observed to be triple nulls, i.e. showing an absence of any amplification product for the SBEIIa genes and therefore having six null alleles for SBEIIa. This confirmed that the triple null seeds could be generated but these seeds had defects that affected germination. Embryos from some of these seeds were excised and cultured using tissue culture media under conditions to promote germination of the embryos. Some embryos germinated successfully, resulting in green plantlets. However, when these plantlets were transferred to soil, they grew poorly and did not produce fertile wheat plants.

From these data, it was concluded that a homozygous triple null mutant seed based on the HIB-generated deletion mutations, and plantlets derived from these seed and having six null SBEIIa alleles and entirely lacking SBEIIa, were recoverable from these crosses, but were affected in germination and growth, indicating an essential role for some SBEIIa in these processes. In contrast, the double null mutants for SBEIIa which were heterozygous for the third null allele and therefore having five null SBEIIa alleles were recovered, grew normally and were fertile, albeit with reduced fertility.

Protein Expression Analysis of Line S28.

SBEIIa protein expression in developing endosperms obtained from one whole spike from an S28 plant was analysed by Western blotting using a SBEIIa specific antibody. All 15 endosperms in the spike showed a pattern lacking both A and D genome isoforms of SBEIIa (AD double null) with only one SBEIIa band present, expressed from the B genome. Out of the 15 endosperms, 7 had a B genome SBEIIa expression level considerably lower than the others and that of the control line, NB1. Based on the band intensity, the SBEIIa expression in each endosperm was quantitated.

The remaining starch granules from the endosperms were purified using 90% Percoll. Following resuspension in 200 µl water, the granules were examined microscopically. It was observed that all endosperms having an expression level of SBEIIa which was less than about 36% of the wild-type had starch granules with distorted morphology typical of a high amylose phenotype. A range of SBEIIa protein expression levels were observed in the developing grains from one spike from an S24 plant, down to less than 5% of wild-type. Endosperms with the lower levels of SBEIIa also showed altered starch granule morphology; the phenotypes were therefore completely correlated in this experiment. SBEIIb expression levels in all these endosperms were also analysed using a SBEIIb specific antibody. The results clearly showed that there was a concomitant reduction in the SBEIIb expression corresponding to the reduction in SBEIIa expression.

Discussion.

The analysis of the seed from plants with the A1B2 mutant genotype (summarised in Table 18) having four mutant SBEIIa alleles indicated that the amylose content was elevated only slightly for that genotype, yielding an amylose level of less than 40%. In comparison, the data from the S14, S22, S24 and S28 seeds demonstrated that the addition of the fifth SBEIIa mutant allele elevated the amylose level to about 67%. Accordingly, the increase in number from four SBEIIa null alleles to a minimum of five mutant SBEIIa alleles was critical to increasing the amylose level to greater than 50% (w/w), indeed greater than 60% (w/w). This conclusion fitted with the predictions made from the data in Example 2. The observed relationship of the allelic composition to the amylose content indicated that the total number of SBEIIa mutant alleles in the plant was important in determining the amylose content (Table 18). It was also concluded that the number of

SBEIIb mutant alleles also played a role, although less important than the number of SBEIIa mutant alleles.

It was also concluded that homozygous triple null mutant seeds and plantlets having six null SBEIIa alleles and entirely lacking SBEIIa could be generated from the single null mutants containing HIB-generated deletions, but these were affected in germination and growth, indicating an essential role for some SBEIIa in these processes. In contrast, the double null mutants for SBEIIa which were heterozygous for the third null allele and therefore having five null SBEIIa alleles were recovered, grew normally and were fertile.

EXAMPLE 8

Further Attempts to Produce Triple-Null Mutants Entirely Lacking SBEIIa or SBEIIb

The observed inability to generate a triple null mutant completely lacking SBEIIa in the Example above may have been dependent on the particular mutant plants used as parents in the crossing. To test this, a second set of crosses using additional parental mutants, also obtained from the HIB-mutagenesis, was carried out, summarised in Table 19. The F2 seeds from 38 crosses were harvested and DNA extracted. At least 96 DNA each from 25 crosses, 12 of which are from crosses aimed at producing an A1B2D2 genotype (triple null mutant) but using different parental lines than described in Example 7, was screened by PCR to determine the trend of segregation. No viable triple nulls were identified from any of these crosses. Recovery of the double nulls also varied depending on the cross, but in most cases the expected genotypes were obtained. F2 seeds from six of the A1B2D2 crosses were also screened microscopically to identify seeds having a high amylose phenotype. Such seeds were identified at a moderate frequency.

Screening of Seeds from the A2B2D2 Cross, 08/mm-1.

Among the crosses listed in Table 19, 12 were crosses between a parent with an A2 genotype and a parent with a B2D2 genotype, i.e. both parents were wild-type for all three SBEIIb genes, with the aim of generating triple null SBEIIa mutants having the A2B2D2 genotype. DNA preparations from approximately 672 F2 seeds obtained from the 08/mm-1 cross were screened by PCR. Segregation ratios were distorted from the expected Mendelian ratios, with significantly fewer double nulls identified than expected (Table 20). Nevertheless, all possible combinations of double null mutations were identified in viable seed. No triple nulls of the A2B2D2 genotype were identified amongst the 672 seeds, even though by Mendelian segregation about 10 would have been expected.

In parallel, F2 seeds of the 08/mm-1 cross were screened by microscopy to identify any seeds with a high amylose/distorted starch granule (HA) phenotype. Of 576 F2 seeds that were screened, no seeds were identified with the HA phenotype. This population of seeds should have included a low frequency of seeds having 5 mutant SBEIIa alleles, being homozygous mutant in two genomes and heterozygous mutant/wild-type in the third genome for SBEIIa. The observed lack of seeds with a HA phenotype in the A2B2D2 cross indicated that 5 mutant SBEIIa alleles, in the absence of any SBEIIb mutant alleles, did not appear to be sufficient to provide a high amylose (>50% amylose) phenotype. That is, a reduction in SBEIIb levels relative to wild-type in addition to the greatly reduced SBEIIa level in the context of 5 mutant SBEIIa alleles and one wild-type SBEIIa allele, or an equivalent level of SBEIIa activity in an endosperm having partial loss of function mutations in one or more SBEIIa genes, was needed to provide greater than 50% amylose.

Screening of F2 seeds from eleven additional crosses between single SBEIIa mutant parents (wild-type for SBEIIb) and double SBEIIa mutant parents on the other two genomes also did not identify any viable triple null mutant seed of the A2B2D2 genotype.

Crosses Involving Type 3 Mutations.

Crosses involving Type 3 mutations were carried out with the aim of finding homozygous mutants having two, four or six mutant SBEIIb alleles combined with four mutant SBEIIa alleles, and determining the phenotype of the resultant plants and its grain. Table 21 summarises the results of screening of crosses involving Type 3 mutations. Triple nulls were identified from A3B3D3 and A3B2D2 crosses, both of which showed wild type starch granule morphology.

EXAMPLE 9

Further Screening for High Amylose Mutants

In further attempts to produce triple null SBEIIa mutants from identified single mutants, an altered strategy was adopted. This strategy added the step of some initial backcrosses of the single mutants after their identification, in order to remove unlinked and unrelated mutations from the M2 plants having the single SBEIIa mutations. This was included to reduce the effect of the mutated background, due to the high level of mutagenic treatment used, which would have produced additional mutations in the plants independent of the desired SBEIIa mutations that could have detrimental effects when the mutations were combined. These initial backcrosses were carried out by crossing the M2 mutants with plants of either winter wheat cultivar Apache or spring wheat cultivar Chara.

Initially, 13 crosses were performed to combine mutations on all three genomes, and molecular analysis was done on DNA from 21,400 F2 half seeds, with the second half of each seed retained to preserve the line. A preliminary screening to detect mutations used dominant SSR markers which were genome specific for SBEIIa or SBEIIb. From this, 21 seeds were identified as being putative triple null mutants and 793 seeds as putative double mutants (Table 22) by the absence of genome specific amplification products.

Q-PCR TaqMan-Based Assays of Wheat Seed Genotypes.

The first round of screening using dominant markers as described above could not distinguish between seeds that were heterozygous or homozygous wild-type for any one SBEIIa gene. A TaqMan-based PCR assay was therefore developed to distinguish heterozygotes and homozygotes for the SBEIIa gene on the third genome, and to confirm the genotypes from the initial screening. Because the TaqMan analysis was done on half seeds and because wheat endosperm is triploid (3n) for each genome, two types of profiles were possible for heterozygous endosperm for the wild-type SBEIIa allele on the third genome, either 2n, where the wild-type allele was provided by the maternal parent, or 1n, where the wild-type allele was provided by the paternal parent through the pollen. Q-PCR TaqMan-based Assays used the Applied Biosystems 7900HT Fast Real Time PCR System (ABI, Foster City, Calif.) to detect the copy number of the SBEIIa gene on the third genome of putative double mutant wheat seeds. The assays used genomic DNA extracted from half seeds by magnetic bead methods (Nucleomag, Cat Ref No. 744 400.24). DNA was loaded on 384-well plates and duplex Q-PCR reactions were performed in duplicate for each plate. The PCR reactions were designed to amplify a 65

bp fragment from exon 21 of the SBEIIa genes using the primers SBE2a QPCRABDF4 (forward primer): 5'-ACGATGCA CTCTTTGGTGGAT-3' (SEQ ID NO: 31) and SBE2a QPCRABDR4 (reverse primer): 5'-ACTTACGGTTGTGAAGTAGTCGACAT (SEQ ID NO: 32). The probe used to deliver the fluorescent signal during Q-PCR reactions was SBE2a QPCRABDS4 (TaqMan probe MGB, FAM) 5'-CAGCAGGCTTGATCAT-3' (SEQ ID NO: 33). A sequence from an endogenous gene, GamyB, was used as an internal control to normalize the signal value of each sample, using the primers GamyB1F (primer forward): 5'-GATCCGAATAGCTGGCTCAAGTAT-3' (SEQ ID NO: 34) and GamyB2R (primer reverse): 5'-GGAGACTGCAGGTAGGGATCAAC-3' (SEQ ID NO: 35). Reaction conditions were as follows: "hot start" (95° C., 10 min) followed by 40 cycles of denaturation (95° C., 15 sec), annealing (58° C., 60 sec). Reaction products were analysed using Relative Quantification manager software integrated to the 7900HT Fast Real Time PCR System.

Using this TaqMan assay, all of the 21 putative triple null mutants were confirmed to be double nulls, not triple nulls. The incorrect identification in the initial screening was thought to be due to false negatives, perhaps caused by poor template DNA quality. When 14 of the seeds were examined for starch granule morphology by light microscopy, all 14 were observed to have a wild-type granule phenotype, which was consistent with the seeds being double null mutants, not triple null mutants. The assays also identified a few putative double mutant seeds that were 2n heterozygous on the third genome, from crosses M76, M77, M82, M83 and M86. However, those results need to be confirmed as it was difficult to distinguish the 2n heterozygous genotype from the 3n homozygous genotype, even in the presence of the double null SBEIIa background. This will be confirmed in the next generation of progeny. The assays also showed that no SBEIIa double null mutants that were heterozygous mutant SBEIIa/wild-type on the third genome were obtained from crosses M79, M81, M74, M75, M78 and M80. The crosses M84 and M85 gave the highest number of clearly identified homozygous double null SBEIIa mutants which were good candidates for being 1n heterozygotes (mutant SBEIIa/wild-type) on the third genome. Some 2n heterozygotes were also identified but need to be confirmed.

In these crosses, the numbers of single and double null SBEIIa mutants was lower than the frequency expected from Mendelian segregation. This distortion of segregation was further studied. Where the expected frequency of homozygous single mutants should have been 25%, in some crosses the frequency was much lower, ranging from 1% to 2.5%. The number of double homozygous mutants in the progeny of crosses to produce triple null mutants should theoretically be around 6% (1/4*1/4) per combination (6% AB, 6% AD, and 6% AB). The actual number of double mutants identified was much lower and ranged from 0 to 5.2%. This suggested that some combinations of mutations were detrimental to the plant, for example to seed development, leading to a lower recovery of combinations of mutations than expected. Two crosses, M74 and M75, gave the lowest frequencies compared to the expected. It was noted that the parents used in those crosses had not been backcrossed with Apache or Chara before the crosses were performed, suggesting that additional, unrelated mutations in the parents arising from the mutagenic treatment may have had a role in the distortion of segregation ratios. Even for crosses M76 and M86 which gave a higher number of single mutants, the frequency of double null mutants was low, in particular for some combinations. For example, for cross M76 the frequencies of single nulls in the D genome and the A genome were 23% and 17%, respectively, while the frequency of the double null mutants in both the A and D genomes was only 0.8%. This suggested that some combinations of SBEIIa mutations were less favorable to the plant than others, and consequently counter-selected. The proportion of mutants containing five mutant SBEIIa alleles (double nulls which were heterozygous mutant on the third genome) was also very low. The expected frequency would be 9% (1/4*1/4*1/2*3) while the highest observed percentage was 1.1% for the M84 and M85 crosses.

Correlation between frequencies of homozygous single and double mutants in M74 and M75 crosses was quite good for SBEIIa mutations on the A and D genomes (0.789 and 0.558 respectively) while much lower (0.386) for the B genome. A possible explanation would be that one of the parents (19.832 (D1)/20.257(A2) [08/b12]) used in M74 and M75 crosses was a heterozygote in the first place rather than a double homozygous mutant.

Under these conditions, the probability of obtaining a triple null mutant (6 null mutant SBEIIa alleles) was very low and much less than the expected frequency of 1/64. However, selfing of the double mutants which were also heterozygous on the third genome, in particular from the M84 and M85 crosses, is expected to confirm whether the triple null mutants are recoverable from these parental mutants. The progeny of the selfed plants will be analyzed to identify any triple mutant seed.

EXAMPLE 10

Screening for Mutant Wheat Seeds by NIR

A rapid, non-destructive and high throughput method was developed to screen single seeds for a phenotype that was associated with high amylose content. The PCR-based screening methods described in Examples 4-6, while successful in detecting mutants in a population of 15,000 seeds, required DNA preparation from each half seed after cutting each seed manually, and so was time-consuming and tedious. It was determined that Near Infrared Spectroscopy (NIRS) could be used to distinguish between the high amylose and normal amylose phenotypes. Near Infrared Red Spectroscopy (NIRS) is a non destructive technology that has been used to determine some wheat seed properties (McClure, 2003). Wheat single seed NIRS analysis for a waxy starch phenotype (low amylose) has been developed on durum wheat by Delwiche et al. (2006). Dowell et al (2009) developed an automated single seed NIR sorting system to separate waxy, partial waxy and normal durum and hexaploid wheat. To our knowledge, this method has not been used previously to distinguish high amylose seeds in hexaploid wheat.

Development and Validation of Scaled Down Biochemical Reference Method to Measure Apparent Amylose Content in Ground Seed Material.

In order to calibrate NIRS measurements according to apparent amylose content in individual seeds, a mathematical model had to be established to correlate NIRS spectrum data and a biochemical method measuring apparent amylose content on the same sample, in this case single seeds. Standard iodometric methods, for example, the method described in Example 1, routinely use a quantity of seeds which are combined before starch solubilisation, providing bulked (combined) starch which is normally defatted prior to colorimetric measurement of the amylose content based on iodine binding. To be suitable for use for NIRS calibration purposes, this method was modified, simplified and scaled down to allow measurement of apparent amylose content in single seeds, thereby to allow for variation in amylose content between

seeds. The term "apparent amylose content" is used in this context because the modified method did not purify the starch from the ground grain, the lipids interacting with the amylose in the starch were not removed, and the results were expressed as percentage of fresh seed weight rather than as a percentage of the isolated starch from the seed. For these reasons, the values obtained for "apparent amylose content" were much lower than the values obtained using the standard method as described in Example 1.

As a first step, this method was developed by assessing the linearity between the colorimetric response and amylose content using ground wheat grain without starch purification. The high amylose material used for this was wheat grain transformed with the hp5'-SBEIIa construct and having reduced SBEIIa (WM, Line 85.2c, see Example 2) and wheat with the normal amylose level which was a wild-type wheat (WMC) grown at the same time and under the same conditions. Ground WM grain contained about 80% amylose as determined by the standard method of Example 1, while ground WMC grain had an amylose content of about 25%. Samples with different ratios of WM to WMC were prepared from ground seed material but not further purified. Approximately 17 mg samples were used for the assay. The WM and WMC mixtures were weighed accurately into 1.5 ml microcentrifuge tubes. To solubilise the starch in the samples, 1 ml of DMSO was added per 17 mg of sample and then the mixtures heated in a 95° C. water bath for 90 min with occasional vortexing. A 10 μl aliquot from each mixture was added to 1.98 ml of water and treated with 10 μl 0.3% I_2+3% KI in 0.01N NaOH solution. The absorbance of each mixture was measured at 605 nm and absorbance values were converted to percent amylose using a standard curve. The standard curve was made using maize amylopectin (Sigma catalogue No. A7780) and potato amylose (Sigma, A0512) in ratios from 0% to 100% amylose and treated the same way as the ground wheat samples.

The results showed a linear relationship between the level of WM incorporation and the apparent amylose content, showing that the simplified iodometric method could be used for NIRS calibration and that starch purification was not needed for this purpose.

Testing the Biochemical Reference Method to Measure Apparent Amylase Content in Half Seeds.

Seeds from the WM and WMC (control) lines obtained from field trial experiments conducted in Arizona and Washington were used for this testing. In total, 47 half seeds with embryos removed were individually placed in 1.5 ml microcentrifuge tubes and weighed accurately before addition of 0.6 ml of DMSO to each. The tubes were incubated in a waterbath at 95° C. for 20 min after which the samples were crushed in the tubes using a glass rod. The volume of each mixture was adjusted to precisely 1 ml of DMSO per 17 mg of sample after which the tubes were incubated at 95° C. in a waterbath for another 70 min with occasional vortexing. Apparent amylose was measured by taking 10 μl aliquots of each mixture and treating them with 10 μl 0.3% I_2+3% KI in 0.01N NaOH solution and diluted to 2 ml with H_2O, as before. Absorbance of each sample was measured at 605 nm and absorbance values were converted to percent "apparent amylose" using a standard curve as described above.

Using this method, the apparent amylose content of WM seeds ranged from 20% to 41% (average 27%) while the apparent amylose content of WMC seeds ranged from 7.5% to 17% (average 11.4%). The reasons why these values were much lower than the amylose content as determined by the method of Example 1 are described above. This simplified method therefore allowed seeds with high amylose to be distinguished from those with wild-type amylose content.

NIRS Calibration.

Single seed NRS scans on WM and WMC seeds were obtained using a Multi Purpose Analyser (MPA) NIRS spectrometer (Bruker Optics, F-77420 Champs Sur Marnes, France). Each seed was placed at the bottom of a glass tube wrapped with aluminium foil and scanned twice. Spectra were recorded using a Bruker MPA Multi-Purpose-Analyser spectrometer (Bruker Optics) fitted with a fiber probe. Spectra were recorded using 32 scans reference and 16 sample scans over the range 4000-12,500 cm^{-1} at a resolution of 16 cm^{-1} resulting in 1100 data points. The fiber optic probe used was the IN 261 probe for solids.

To determine the correlation between apparent amylose levels and NIR readings, 226 individual WM or WMC seeds with apparent amylose contents ranging from 6 to 44% were analysed. First, duplicate NIRS spectra were acquired for each seed, after which the apparent amylose content was biochemically measured for each seed according to the method described above. Spectral outliers (6 samples) were identified as spectra that were abnormal compared to the spectra of the entire data set and eliminated, and the remaining spectra analysed with Normalisation Min-max pre-treatment. The Partial Least Square software with full (one out) cross validation was used to create the model. The spectral window used for the model development was 9827-7150 cm^{-1} and 6271-4481 cm^{-1}. The number of PLS factors used to develop the calibration was 14. The accuracy of the calibration model was expressed by the standard error of cross validation (SECV) and the coefficient of determination (R^2). The efficiency of a calibration was shown by the RPD which is the ration of the standard error of prediction (RMSECV) to the standard deviation of the reference data of the set.

A positive correlation (R^2=0.702) was obtained between the biochemical data and the NIR spectral data (FIG. **15**). It was concluded that the model was robust enough to distinguish high amylose wheat seeds from normal amylose wheat seeds, but not yet accurate enough to precisely measure the amylase content in any one seed. The method was therefore capable of screening a very large population of seeds to enrich for grains with high amylose phenotype. This was validated as follows.

NIRS Validation.

To validate the NIR method in distinguishing high amylose grain and control grain, 60 more WM seeds and 34 WMC seeds were scanned twice by NIR and the predicted apparent amylose contents calculated. When the apparent amylose values so determined were plotted to obtain the distribution profile for the WM and WMC populations, it was seen that the two groups were mostly separated with a slight overlap (FIG. **16**). According to these results, seeds having a predicted apparent amylose phenotype determined by NIRS equal to or above 30% could be considered as good candidates to be high amylose seed.

NIRS Screening of F2 Seeds from Wheat Crosses.

NIRS screening was carried out to detect mutant seeds having high amylose content. The screening used 2,700 F2 seeds from two different crosses: M80 and M85 which were, respectively: 21.142(B2)/Type 20257(A1) [08/h-111]//Type I-19.832 (D1)/CHARA and 5.706 (D2)/21.668 (B2)/120.257 (A1)/CHARA. The screening was therefore aimed at identifying seeds with an A1B2D1 or A1B2D2 genotype, respectively. Two NIRS spectra were recorded per seed as described above

Seeds which gave a predicted apparent amylose value above 34% in at least one of the two duplicate screenings were

first selected for further analysis. Out of the 2,700 seeds, 27 seeds were selected and were next assessed by light microscopy to determine the starch granule morphology. Each seed was carefully scraped to preserve the embryo, yet obtain enough endosperm material to be examined. Four seeds of the 27 were observed to have distorted starch granule morphology. These four seeds happened to have had the highest predicted apparent amylose content from the NIR screening and were the only ones where both predicted apparent amylose values were above 30%. The other 23 seeds showed normal (wild-type) granule morphology.

Molecular Data on Seeds Selected by NIRS Screening.

PCR analysis was carried out on the four seeds to determine the SBEIIa genotype of each. Initial assays used dominant PCR markers which showed the presence or absence of each SBEIIa gene on the three genomes. Three of the seeds were shown to be double null mutants while the fourth was a putative triple null mutant. However, when tested further with a co-dominant PCR marker (see below), all of the four seeds were shown to be double null mutants for SBEIIa (i.e. lacking SBEIIa in two genomes) and heterozygous for a mutant SBEIIa gene on the third genome. Therefore, these seeds contained 5 mutant SBEIIa alleles and at least two mutant SBEIIb alleles.

When the embryo from each seed was placed under conditions to germinate, none of them germinated successfully, perhaps because they were too damaged or the combination of mutations was too detrimental.

In order to try to identify more candidates, further NIRS screening was performed on more F2 progeny seeds from the M80 and M85 crosses, with less stringent selection of candidate seeds. The selection criterion for the second screen was that one of the predicted apparent amylose values had to be above 30% and the second one at least 23%. A new set of 22 seeds was selected for starch granule evaluation by light microscopy. Out of those 22 candidates, 1 seed, BD85; 9F08 (P279F08-834), showed a distorted starch granule phenotype. This mutant was further analysed by PCR and shown to be a double null SBEIIa mutant on the A and B genomes and heterozygous for the mutant SBEIIa gene on the D genome. It was successfully germinated for multiplication.

EXAMPLE 11

Detection of Alleles of Starch Branching Enzyme with Altered Starch Binding Affinity

Populations of mutagenised wheat grains, produced by treatment with the chemical mutagens sodium azide or EMS were screened to identify mutants which had point mutations in SBEIIa genes and therefore potentially reduced, but not abolished, SBEIIa-A, -B or -D activity, or SBEIIb-A, -B or -D activity (partial mutants) relative to wild-type wheat. Screening for mutants was based on measuring the amount of the SBEIIa or SBEIIb proteins by using Western blotting with antibodies specific for SBEIIa or SBEIIb (see Example 2), or by affinity-based techniques, as follows. This screening was also expected to detect mutants with point mutations which lacked SBEIIa-A, -B, or -D activity entirely as well as the mutants with partial activity.

Native gel electrophoresis of protein extracts from grain including starch branching enzymes through a polyacrylamide matrix containing glycogen, amylopectin, amylose or β-limit dextrin (affinity gel electrophoresis) provides a method for identifying alleles of SBEIIa or SBEIIb which encode SBEIIa or SBEIIb with altered starch binding capacity. Given that the active site of starch branching enzymes contains a starch binding site, SBEII polypeptides with altered binding efficiency are likely to have alterations in catalytic rate and/or affinity. In particular, polypeptides with reduced binding efficiency were expected to have reduced SBEII activity.

The following methods were used, based on Morell et al., (1997); and Kosar-Hashemi et al., (2006) with some modifications.

Preparation of Proteins.

Soluble proteins were extracted by homogenising the isolated endosperms from developing seeds (about 15 days post-anthesis) in 50 mM phosphate buffer, pH 7.5 containing 5 mM EDTA, 5 mM DTT, 0.4% protease inhibitor cocktail and 20% glycerol. After centrifugation at 14,000 g for 10 min the supernatant was used for the gel electrophoresis. Protein concentration in the extracts was estimated using a Coomassie Plus Protein Assay Reagent.

Affinity Electrophoresis.

In a two-dimensional (2D) affinity electrophoresis technique for separating SBEIIa protein isoforms, aliquots (40 or 100 μg) of the protein extracts were loaded onto the first dimension gel, a non-denaturing polyacrylamide gel cast in a Hoefer SE600 vertical 16 cm slab gel unit. The resolving component of the second dimension gel was a 6% non-denaturing gel (14×16 cm or 16×16 cm, 1.5 mm thickness) containing 10% glycerol with an appropriate amount of polysaccharide target (amylopectin, β-limit dextrin or glycogen) immobilised within the gel structure. A stacking gel (polysaccharide-free) was poured to 1 cm from the top of glass plates forming using a comb to form wells. Gels were run overnight at 4° C. at constant voltage (100V for glycogen and β-limit dextrin and 135V for amylopectin containing gels).

Alternatively, a one dimensional system was used to separate SBEIIa proteins in which protein extracts (20 μg) were loaded onto a non-denaturing polyacrylamide gel. The resolving component of the gel was a 6% non-denaturing gel containing 10% glycerol with 0.15% of β-limit dextrin immobilised within the gel structure, while the stacking gel was polysaccharide-free. Gels were run at 4° C. at constant current of 20 mA per gel and maximum voltage of 200V.

SBEIIb proteins can also be separated on a Bis-Tris 4-12% gradient gel (Invitrogen). The gel is run at 4° C. at constant current of 20 mA per gel and maximum voltage of 200V.

Immunological Detection.

For immunochemical detection of the SBEII proteins following electrophoresis, the proteins were transferred from the gels to nitrocellulose membranes using a TE 70 PWR semi-dry transfer unit (Amersham Biosciences). The transfer buffer contained 39 mM glycine, 48 mM Tris, 0.0375% SDS and 20% methanol. Transfer was carried out for 1-1.5 h with a constant current of 0.8 mA/cm^2. The membrane was blocked with 5% skim milk prior to Western blotting using primary rabbit polyclonal antibody specific for wheat SBEIIa.

The migration patterns of the SBEII isoforms encoded by the homeoalleles from the wheat A, B and D genomes showed differences between different wheat varieties when analysed by the one-dimensional affinity gel electrophoresis method. In some varieties, clear separation of the A, B and D homeoforms was possible, allowing the simple scoring of polymorphisms in mutagenised populations from those varieties. For example, affinity gel electrophoresis of protein extracts from endosperms of the wild-type wheat varieties Sunstate and NB1 showed a clear separation of the SBEIIa-A, -B and -D isoforms. Branching enzyme alleles with a reduced affinity for starch migrated a greater distance through the polysaccharide-containing polyacrylamide gel than the respective

native homeoalleles. Lines containing alleles with reduced expression or an absence of expression of a particular homeoallele were identified by presence/absence of a band in homozygous state and through densitometry to measure band intensity in heterozygous lines. To validate this method, SBEIIa- and SBEIIb-mutant plants which were identified by genotypic analysis (Example 6) were confirmed to be lacking specific SBEIIa or SBEIIb proteins by affinity gel electrophoresis, consistent with their genotypes. These experiments validated this protein analysis method for detection of mutants having a reduction in amount or activity of an SBEII isoform.

Screening of a population of 2100 mutagenised wheat lines of the variety Sunstate, treated with sodium azide as described in Zwar and Chandler (1995), using β-limit dextrin affinity gel electrophoresis led to the identification of 18 mutants which had either altered mobility on the affinity gels of one of the SBEIIa proteins (affinity mutants) or null mutants for one of the SBEIIa genes based on a lack of detectable protein encoded by that gene. The dissociation constant (Kd) of starch-enzyme interactions for each of the SBEIIa isoforms in one of the affinity mutants was calculated by measuring the change in enzyme mobility as a function of the β-limit dextrin concentration in a 1-D affinity gel as described in Kosar-Hashemi et al., 2006. This affinity mutant had SBEIIa proteins with the following Kd values: 0.53 g/L, 0.52 g/L and 1.69 g/L for the SBEIIa-A, SBEIIa-B and SBEIIa-D isoforms respectively (FIG. **13**). The higher observed Kd value for the D isoform compared to that of the A and B isoforms indicated a lower, reduced affinity of this isoform for binding to starch, indicating that this line was an affinity mutant for the SBEIIa-D gene. The D-genome isoform (SBEIIa-D) of this line is expected to have a lower enzyme activity, but not total loss of activity, compared to the other two isoforms. This expectation is confirmed by SBEII activity assays in the presence of null alleles of SBEIIa-A and SBEIIa-B.

The SBEIIa single mutants identified from the sodium azide mutagenised Sunstate population were then crossed with the previously identified HIB double null mutants for isolating triple mutants that lack SBEIIa activity from two genomes with total or partial loss of activity from the third genome. Four crosses to isolate A1B2D2, two crosses each to isolate A2B2D2 and A2B2D1 and one cross to isolate A1B2D1 genotypes were performed. Examination of starch granule morphology of F2 seeds from one of the A1B2D2 crosses by microscopy identified seeds with severely distorted starch granules similar to that is found in high amylose starches (at least 70% amylose). The genotype and amylose phenotype of these seeds is confirmed by analysing the SBEIIa alleles in the seeds and progeny and by extracting and analysing starch from the progeny grain. Eight crosses were also performed between affinity single mutants to produce affinity double mutants of SBEIIa. This included crosses generated with the aim of isolating A2B2, A2D2 and B2D2 double affinity mutants. F2 progeny are analysed by the methods described above to identify the double homozygous affinity mutants.

EXAMPLE 12

Properties of Starch Granules and Starch from High Amylose Wheat Grain

Changes in Starch Granule Morphology and Birefringence.

Starch and starch granule properties were examined in the transgenic high amylose wheat described in Example 2. Scanning electron microscopy was used to identify gross changes in starch granule size and structure. Compared to the untransformed control, starch granules from endosperms having reduced SBEIIa expression displayed significant morphological alterations. They were highly irregular in shape and many of the A granules (>10 μm diameter) appeared to be sickle shaped. In contrast, both A and 13 (<10 μm diameter) starch granules from endosperms having reduced SBEIIb expression and unaltered SBEIIa expression were smooth surfaced, spherical or ellipsoid in shape and indistinguishable from wild-type wheat starch granules.

When observed microscopically under polarised light, wild-type starch granules typically show a strong birefringence pattern. However, the birefringence was greatly reduced for granules containing high amylose starch. Less than 10% of the starch granules from lines having reduced SBEIIa expression and 70%-80% amylose content were birefringent when visualized under polarized light. For lines having essentially no SBEIIb expression but with wild-type SBEIIa expression, no change in birefringence was observed compared to non-transformed controls. In both wild-type and SBEIIb-suppressed lines, approximately 94% of the starch granules exhibited full birefringence. The data is given in Table 23. Loss of birefringence therefore correlated closely with high amylose content.

Amylose Content of Transgenic Wheal Grain.

The amylose content of transgenic wheat grain was assayed by two independent methods, namely an iodometric method and a size exclusion chromatography (SEC) method. The iodometric determination of amylose content was based on measuring the colour change induced when iodine bound to linear regions of α-1,4 glucan, with reference to a standard curve generated using known concentrations of purified potato amylose and amylopectin, as described in Example 1. The size exclusion chromatography method was based on the separation, by column chromatography, of amylose and amylopectin which had not been debranched, followed by measurement of the starch concentration in the fractions eluted from the column. Three genotypes of grain were analysed. Firstly, plants transformed with the hp-SBEIIa construct and having very low levels of SBEIIa expression; secondly, plants containing the hp-SBEIIb construct and having no detectable expression of SBEIIb but wild-type for SBEIIa; and thirdly, the non-transformed wild-type control (NB1). Grain from the plants lacking SBEIIb expression (008) had an amylose content of 27.3% determined by the iodometric method and 32% by the SEC method. This was not significantly different to the amylose content of grain from non-transformed control line NB1 (31.8% iodometric, 25.5% SEC). However, in grain having the reduced SBEIIa expression (line 087) the amylose content was significantly elevated (88.5% iodometric, 74.4% SEC). The difference in these two figures for line 087 was thought to be the presence of some "intermediate material" which binds iodine much like amylose and was measured in the iodometric assay as amylose but was separated in the column chromatography with the larger amylopectin.

Chain Length Distribution of Starch by FACE.

Chain length distribution of isoamylase de-branched starch was determined by fluorophore assisted carbohydrate electrophoresis (FACE). This technique provides a high resolution analysis of the distribution of chain lengths in the range from DP 1 to 50. From the molar difference plot in which the normalized chain length distribution of the non-transformed control was subtracted from the normalized distribution of the transgenic lines, it was observed that there was a marked decrease in the proportion of chain lengths of DP 6-12 and a corresponding increase in the chain lengths greater than DP12 in starch from grain having reduced SBEIIa expression.

No statistically significant alteration in the chain length distribution of starch from hp-SBEIIb lines was observed when compared to wild-type.

Molecular Weight of Amylopectin and Amylose.

Molecular weight distribution of starch was determined by size exclusion-HPLC (SE-HPLC). The HPLC system comprised of a GBC pump (LC 1150, GBC Instruments, Vic, Australia) equipped with Auto Sampler (GBC, LC1610) and Evaporative Light Scattering Detector (ELSD) (ALLTech, Deerfield, USA). The Ultrahydrogel™ 1000 column, Ultrahydrogel™ 250 column and guard column (7.8 mm×300 mm, Waters, Japan) were used and maintained at 35° C. during HPLC operation. Ammonium acetate buffer (0.05 M; pH 5.2) was used as the mobile phase at a flow rate of 0.8 mL min^{-1}.

The molecular weight of amylopectin in the starch of the reduced SBEIIa grain appeared to be much lower than that of amylopectin in the starches of NB1 (wild-type, non-transgenic) and the reduced SBEIIb grain (peak position of 7166 kDa versus 45523, 43646 kDa). In contrast, the molecular weight of amylose from the reduced SBEIIb grain was not significantly different compared to that of wild-type grain from non-transformed variety NB1. The data is in Table 24.

Total Starch Content in Endosperm of Wheat with Reduced SBEIIa Expression.

Analysis of total starch content in grain as a percentage of grain weight revealed a slight reduction in the endosperm starch content of the hp-SBEIIa line (43.4%) compared to 52% in the control and 50.3% in hp-SBEIIb line (Table 23). This indicated that there was some reduction in total starch synthesis when SBEIIa expression was reduced by the inhibitory construct.

Starch Swelling Power (SSP).

Starch swelling power gelatinized starch was determined following the small scale test of Konik-Rose et al., (2001) which measured the uptake of water during gelatinization of starch. The estimated value of SSP was significantly lower for starch from the reduced SBEIIa line with a figure of 3.51 compared to starch from the control (9.31) and reduced SBEIIb grain (10.74) (Table 23).

Starch Pasting Properties.

Starch paste viscosity parameters were determined using a Rapid Visco Analyzer (RVA) essentially as described in Regina et al., (2004). The temperature profile for the RVA comprised the following stages: hold at 60° C. for 2 min, heat to 95° C. over 6 min, hold at 95° C. for 4 min, cool to 50° C. over 4 min, and hold at 50° C. for 4 min. The results (Table 25) showed that the peak and final viscosities were significantly lower in starch from the reduced SBEIIa grain compared to the control wheat starch.

Starch Gelatinisation Properties.

Gelatinisation properties of starch were studied using differential scanning calorimetry (DSC) as described in Regina et al., (2004). DSC was carried out on a Perkin Elmer Pyris 1 differential scanning calorimeter. Starch and water were premixed at a ratio of 1:2 and approximately 50 mg weighed into a DSC pan which was sealed and left to equilibrate overnight. A heating rate of 10° C. per minute was used to heat the test and reference samples from 30 to 130° C. Data was analysed using the software available with the instrument. The results (Table 26) clearly showed a delayed end of gelatinisation temperature (72.6° C.) for starch from the reduced SBEIIa grain compared to the control (66.6° C.). The peak gelatinisation temperature was also higher in the reduced SBEIIa starch (63.51° C.) compared to the control starch (61.16° C.).

EXAMPLE 13

Analysis of High Amylose Wheat Flour During Processing

Pressure Processing Studies in Collaboration with CSIRO Food and Nutritional Sciences, Werribee.

Structural characterisation of high amylose wheat starches in comparison with native starch was carried out using Small Angle X-ray Scattering (SAXS). The study was designed to include a) characterising raw wheat flour and b) real-time analysis of the gelatinisation process while pressure cooking the flour or starch samples at temperatures of greater than 100° C. and c) Structural changes on cooling over a period of 0 to 10 days, and retrogradation. The study used wheat flour samples of varying amylose content ranging from about 25% (wild-type) to about 75%, increasing in increments of about 10%.

Three sets of flour samples were included in the experiments. Firstly, with pure lines without pooling from a high amylose wheat from the reduced SBEIIa lines, a medium level amylose wheat line AC45.1 which was transformed with the hp-combo construct having about 50% amylose (Example 2) and from the control wheat (NB1). Secondly, with pooled wheat material from transformed lines as described in Example 2, pooling samples in increments of 10% increasing amylose content. Thirdly, comparing flour from different species including wheat (high amylose, wild-type, and wheat lacking SSIIa), barley (wild-type, high amylose by reduced SBEIIa and SBEIIb, and high amylose by reduced SSII), and high amylose maize. The results from the resistant starch analysis on the pooled wheat material with a range of amylose content revealed a linear increase in resistant starch from an amylose content of ≥40%.

EXAMPLE 14

Production of Breads and Other Food Products

One of the most effective ways of delivering a grain such as high amylose wheat into the diet is through bread. To show that the high amylose wheat could readily be incorporated into breads and to examine the factors that allowed retention of bread making quality, samples of flour were produced, analysed and used in baking. The following methods were employed.

Methods.

Wheat grains were conditioned to 16.5% moisture content overnight and milled with either a Buhler laboratory scale mill at BRI Ltd, Australia, or using a Quadromat Junior mill followed by sieving, to achieve a final particle size of 150 pin. The protein and moisture content of the samples were determined by infrared reflectance (NIR) according to AACC Method 39-11 (1999), or by the Dumas method and air-oven according to AACC Method 44-15 A ($AACC_5$ 1999).

Micro Z-Arm Mixing.

Optimum water absorption values of wheat flours were determined with the Micro Z-arm Mixer, using 4 g of test flour per mix (Gras et al., (2001); Bekes et al., (2002). Constant angular velocity with shaft speeds for the fast and slow blades of 96 and 64 rpm, respectively, were used during all mixes. Mixing was carried out in triplicate, each for 20 minutes. Before adding water to the flour, the baseline was automatically recorded (30 sec) by mixing only the solid components. The water addition was carried out in one step using an automatic water pump. The following parameters were determined from the individual mixing experiments by taking the

averages: WA %—Water Absorption was determined at 500 Brabender Unit (BU) dough consistency; Dough Development Time (DDT): time to peak resistance (sec).

Mixograms.

To determine optimal dough mixing parameters with the modified wheat flour, samples with variable water absorption corresponding to water absorption determined by the Micro Z-arm mixer, were mixed in a 10 g CSIRO prototype Mixograph keeping the total dough mass constant. For each of the flour samples, the following parameters were recorded: MT—mixing time (sec); PR—Mixograph peak resistance (Arbitrary Units, AU); BWPR—band width at peak resistance (Arbitrary Units, AU); RBD—resistance breakdown (%); BWBD—bandwidth breakdown (%); TMBW—time to maximum bandwidth (s); and MBW—maximum bandwidth (Arbitrary Units, AU.).

Micro Extension Testing.

Dough extensibility parameters were measured as follows: Doughs were mixed to peak dough development in a 10 g prototype Mixograph. Extension tests at lcm/s were carried out on a TA.XT21 texture analyser with a modified geometry Kieffer dough and gluten extensibility rig (Mann et al., 2003). Dough samples for extension testing (−1.0 g/test) were moulded with a Kieffer moulder and rested at 30° C. and 90% RH for 45 min. before extension testing. The R_Max and Ext_R-max were determined from the data with the help of Exceed Expert software (Smewing, The measurement of dough and gluten extensibility using the SMS/Kieffer rig and the TA.TX2 texture analyzer handbook, SMS Ltd: Surrey, UK, 1995; Mann, (2002).

An illustrative recipe based on the 14 g flour as 100% was as follows: flour 100%, salt 2%, dry yeast 1.5%, vegetable oil 2%, and improver (ascorbic acid 100 ppm, fungal amylose 15 ppm, xylanase 40 ppm, soy flour 0.3%, obtained from Goodman Fielder Pty Ltd, Australia) 1.5%. The water addition level was based on the micro Z-arm water absorption values that were adjusted for the full formula. Flour (14 g) and the other ingredients were mixed to peak dough development time in a 35 g Mixograph. The moulding and panning was carried out in a two staged proofing steps at 40 C at 85% RH. Baking was carried out in a Rotel oven for 15 min at 190° C. Loaf volume (determined by the canola seed displacement method) and weight measurements were taken after cooling on a rack for 2 hours. Net water loss was measured by weighing the loaves over time.

The flour or wholemeal may be blended with flour or wholemeal from non-modified wheats or other cereals such as barley to provide desired dough and bread-making or nutritional qualities. For example, flour from cvs Chara or Glenlea has a high dough strength while that from cv Janz has a medium dough strength. In particular, the levels of high and low molecular weight glutenin subunits in the flour is positively correlated with dough strength, and further influenced by the nature of the alleles present.

Flour from transgenic wheat lines having reduced SBEIIa were used at 100%, 60% and 30% addition levels. e.g. either all the flour came from the various wheat lines or 60% or 30% were added to the Baking Control (B. extra) flour. Percentages are of total flour in the bread formulation. Four transgenic wheat lines were used as follows: 072 (reduced SBEIIa), 212 (a wheat line derived from the cross, reduced SBEIIa×SBEI triple null wheat), H7 (a wheat line derived from the cross, reduced SBE IIa×SSIIa triple null wheat) and 008 (reduced SBEIIb) were tested along with a non transformed control wheat (NB1). All wheats were milled in a Brabender Quadramat Junior mill. All blends had water absorptions determined on 4 g Z-arm mixer and optimal mixing times determined on 10 g Mixograph as described above. These conditions were used in preparing the 10 g test bake loaves.

Mixing Properties.

Apart from the control lines (Baking Control, NB1 and 008) all other wheat lines gave greatly elevated water absorption values (FIG. **17**(*a*)). Lines 212 and 072 gave increasing water absorption values with increasing addition levels, including up to a high of 95% water absorption at 100% addition of 212 flour. Increased incorporation levels of flour from these lines also lead to a decrease in the optimal Mixograph mixing times (FIG. **17**(*b*)). As with the water absorption data, the non-control lines showed a strong reduction in specific loaf volume (loaf volume/loaf weight) with increasing levels of addition. The effect was particularly strong for the 212 line.

These studies show that breads with commercial potential, including acceptable crumb structure, texture and appearance, could be obtained using the high amylose wheat flour blended with control flour samples. Furthermore, high amylose wheats are used in combination with preferred genetic background characteristics (e.g. preferred high and low molecular weight glutenins), the addition of improvers such as gluten, ascorbate or emulsifiers, or the use of differing bread-making styles (e.g. sponge and dough bread-making, sour dough, mixed grain, or wholemeal) to provide a range of products with particular utility and nutritional efficacy for improved bowel and metabolic health.

Other Food Products:

Yellow alkaline noodles (YAN) (100% flour, 32% water, 1% Na_2CO_3) were prepared in a Hobart mixer using the standard BRI Research Noodle Manufacturing Method (AFL 029). Noodle sheet was formed in the stainless steel rollers of an Otake noodle machine. After resting (30) the noodle sheet was reduced and cut into strands. The dimensions of the noodles were 1.5×1.5 mm.

Instant noodles (100% flour, 32% water, 1% NaCl and 0.2% Na_2CO_3) were prepared in a Hobart mixer using the standard BRI Research Noodle Manufacturing method (AFL 028). Noodle sheet was formed in the stainless steel rollers of an Otake noodle machine. After resting (5 min) the noodle sheet was reduced and cut into strands. The dimensions of the noodles were 1.0×1.5×25 mm. The noodle strands were steamed for 3.5 min and then fried in oil at 150 C for 45 sec.

Sponge and Dough (S&D) bread. The BRI Research sponge and dough baking involved a two-step process. In the first step, the sponge was made by mixing part of the total flour with water, yeast and yeast food. The sponge was allowed to ferment for 4 h. In the second step, the sponge was incorporated with the rest of the flour, water and other ingredients to make dough. The sponge stage of the process was made with 200 g of flour and was given 4 h fermentation. The dough was prepared by mixing the remaining 100 g of flour and other ingredients with the fermented sponge.

Pasta—Spaghetti. The method used for pasta production was as described in Sissons et al., (2007). Test sample flours from high amylose wheat (reduced SBEIIa) and control wheat (NB1) were mixed with Manildra semolina at various percentages (test sample: 0, 20, 40, 60, 80, 100%) to obtain flour mixes for small scale pasta preparation. The samples were corrected to 30% moisture. The desired amount of water was added to the samples and mixed briefly before being transferred into a 50 g farinograph bowl for a further 2 min mix. The resulting dough, which resembled coffee-bean-size crumbs, was transferred into a stainless steel chamber and rested under a pressure of 7000 kPa for 9 min at 50 C. The pasta was then extruded at a constant rate and cut into lengths

of approximately 48 cm. Two batches of pasta were made for each sample. The pasta was dried using a Thermoline Temperature and Humidity Cabinet (TEC 2604) (Thermoline Scientific Equipment, Smithfield, Australia). The drying cycle consisted of a holding temperature of 25 C followed by an increase to 65 C for 45 min then a period of about 13 h at 50 C followed by cooling to 25 C. Humidity was controlled during the cycle. Dried pasta was cut into 7 cm strands for subsequent tests.

EXAMPLE 15

In Vitro Measurements of Glycaemic Index (GI) and Resistant Starch (RS) of Food Samples

The Glycemic Index (GI) of food samples including the bread made as described herein was measured in vitro as follows: Food samples were homogenised with a domestic food processor. An amount of sample representing approximately 50 mg of carbohydrate was weighed into a 120 ml plastic sample container and 100 μl of carbonate buffer added without α-amylase. Approximately 15-20 seconds after the addition of carbonate buffer, 5 ml of Pepsin solution (65 mg of pepsin (Sigma) dissolved in 65 ml of HCl 0.02M, pH 2.0, made up on the day of use) was added, and the mixture incubated at 37° C. for 30 minutes in a reciprocating water bath at 70 rpm. Following incubation, the sample was neutralised with 5 ml of NaOH (0.02M) and 25 ml of acetate buffer 0.2M, pH 6 added. 5 ml of enzyme mixture containing 2 mg/mL of pancreatin (α-amylase, Sigma) and 28 U/mL of amyloglucosidase from *Aspergillus niger* (AMG, Sigma) dissolved in Na acetate buffer (sodium acetate buffer, 0.2 M, pH 6.0, containing 0.20 M calcium chloride and 0.49 mM magnesium chloride) was then added, and the mixture incubated for 2-5 minutes. 1 ml of solution was transferred from each flask into a 1.5 ml tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred to a new tube and stored in a freezer. The remainder of each sample was covered with aluminium foil and the containers incubated at 37° C. for 5 hours in a water bath. A further 1 ml of solution was then collected from each flask, centrifuged and the supernatant transferred as carried out previously. This was also stored in a freezer until the absorbances could be read.

All samples were thawed to room temperature and centrifuged at 3000 rpm for 10 minutes. Samples were diluted as necessary (1 in 10 dilution usually sufficient), 10 μl of supernatant transferred from each sample to 96-well microtitre plates in duplicate or triplicate. A standard curve for each microtitre plate was prepared using glucose (0 mg, 0.0625 mg, 0.125 mg, 0.25 mg, 0.5 mg and 1.0 mg). 200 ul of Glucose Trinder reagent (Microgenetics Diagnostics Pty Ltd, Lidcombe, NSW) was added to each well and the plates incubated at room temperature for approximately 20 minutes. The absorbance of each sample was measured at 505 nm using a plate reader and the amount of glucose calculated with reference to the standard curve.

The level of Resistant Starch (RS) in food samples including the bread made as described herein was measured in vitro as follows. This method describes the sample preparation and in vitro digestion of starch in foods, as normally eaten. The method has two sections: firstly, starch in the food was hydrolysed under simulated physiological conditions; secondly, by-products were removed through washing and the residual starch determined after homogenization and drying of the sample. Starch quantitated at the end of the digestion treatment represented the resistant starch content of the food.

On day 1, the food samples were processed in a manner simulating consumption, for example by homogenising with a domestic food processor to a consistency as would be achieved by chewing. After homogenising, an amount of food representing up to 500 mg of carbohydrate was weighed into a 125 mL Erlenmeyer flask. A carbonate buffer was prepared by dissolving 121 mg of $NaHCO_3$ and 157 mg of KCl in approximately 90 mL purified water, adding 159 μL of 1 M $CaCl_2.6H_2O$ solution and 41 μL of 0.49 M $MgCl_2.6H_2O$, adjusting the pH to 7 to 7.1 with 0.32 M HCl, and adjusting the volume to 100 mL. This buffer was stored at 4° C. for up to five days. An artificial saliva solution containing 250 units of α-amylase (Sigma A-3176 Type VI-B from porcine pancreas) per mL of the carbonate buffer was prepared. An amount of the artificial saliva solution, approximately equal to the weight of food, was added to the flask. About 15-20 sec after adding the saliva, 5 mL of pepsin solution in HCl (1 mg/mL pepsin (Sigma) in 0.02 M HCl, pH 2.0, made up on day of use) was added to each flask. The mixing of the amylase and then pepsin mimicked a human chewing the food before swallowing it. The mixture was incubated at 37° C. for 30 min with shaking at 85 rpm. The mixture was then neutralised with 5 mL of 0.02M NaOH. 25 mL of acetate buffer (0.2 M, pH 6) and 5 mL of pancreatin enzyme mixture containing 2 mg/mL pancreatin (Sigma, porcine pancreas at 4×USP activity) and 28 U of amyloglucosidase (AMG, Sigma) from *Aspergillus niger* in acetate buffer, 016, were added per flask. Each flask was capped with aluminium foil and incubated at 37° C. for 16 hours in a reciprocating water bath set to 85 rpm.

On day 2, the contents of each flask were transferred quantitatively to a 50 mL polypropylene tube and centrifuged at 2000×g for 10 min at room temperature. The supernatants were discarded and each pellet washed three times with 20 mL of water, gently vortexing the tube with each wash to break up the pellet, followed by centrifugation. 50 μL of the last water wash was tested with Glucose Trinder reagent for the absence of free glucose. Each pellet was then resuspended in approximately 6 mL of purified water and homogenised three times for 10 seconds using an Ultra Turrax TPI 8/10 with an S25N-8G dispersing tool. The contents are quantitatively transferred to a 25 mL volumetric flask and made to volume. The contents were mixed thoroughly and returned to the polypropylene tube. A 5 mL sample of each suspension was transferred to a 25 mL culture tube and immediately shell frozen in liquid nitrogen and freeze dried.

On day 3, total starch in each sample was measured using reagents supplied in the Megazyme Total Starch Procedure kit. Starch standards (Regular Maize Starch, Sigma S-5296) and an assay reagent blank were prepared. Samples, controls and reagent blanks were wet with 0.4 mL of 80% ethanol to aid dispersion, followed by vortexing. Immediately, 2 mL of DMSO was added and solutions mixed by vortexing. The tubes were placed in a boiling water bath for 5 min, and 3 mL of thermostable α-amylase (100 U/ml) in MOPS buffer (pH 7, containing 5 mM $CaCl_2$ and 0.02% sodium azide) added immediately. Solutions were incubated in the boiling water bath for a further 12 min, with vortex mixing at 3 min intervals. Tubes were then placed in a 50° C. water bath and 4 mL of sodium acetate buffer (200 mM, pH 4.5, containing 0.02% sodium azide) and 0.1 mL of amyloglucosidase at 300 U/ml added. The mixtures were incubated at 50° C. for 30 min with gentle mixing at 10 min intervals. The volumes were made up to 25 mL in a volumetric flask and mixed well. Aliquots were centrifuged at 2000×g for 10 min. The amount of glucose in 50 μL of supernatant was determined with 1.0 mL of Glucose Trinder reagent and measuring the absorbance at 505 nm after incubation of the tubes at room temperature in the dark for a minimum of 18 min and a maximum of 45 min.

Bread loaves baked from flour from four transgenic wheat lines, namely 072 (reduced SBEIIa), 212 (a wheat line derived from the cross, reduced SBEIIa×SBEI triple null wheat), H7 (a wheat line derived from the cross, reduced SBEIIa×SSIIa triple null wheat) and 008 (reduced SBEIIb)

were tested along with a non transformed control wheat (NB1) for RS and GI after incorporation levels of 100%, 60% and 30% flour, the remainder 40% or 70% flour being from wild-type grain. Increased incorporation of 212, 072, and H7 flour resulted in significant increases in RS (FIG. **18**(*a*) and reductions in predicted GI (FIG. **18**(*b*)). The magnitude of the changes was greatest when using flour from Line 212. For instance, bread made with 100% addition of this high amylose flour had an RS content of about 10% which represented a 150% increase above that for 30% level of inclusion and a 9-fold increase compared to the NB1 controls. Increasing the extent of incorporation of flour from the 008 lines had no effect on the RS and GI of the resultant loaves and the results were comparable to those of the baking control flour.

EXAMPLE 16

Processing of High Amylose Wheat and Resultant RS Levels

A small scale study was conducted to determine the resistant starch (RS) content in processed grain from the high amylose wheat which had been rolled or flaked. The technique involved conditioning the grains to a moisture level of 25% for one hour, followed by steaming the % ins. Following steaming, the grains were flaked using a small-scale roller. The flakes were then roasted in an oven at 120 C for 35 min. Two roller widths and three steaming timings were used on approximately 200 g of samples from high amylose wheat having reduced SBEIIa (HAW, line 85.2c) and wild-type, control wheat (cv. Hartog). The roller widths tested were 0.05 mm and 0.15 mm. The steaming timings tested were 60', 45' and 35'.

This study showed a clear and substantial increase in the amount of RS in processed high amylose wheat compared to the control (Table 27, FIG. **18**). There also appeared to be some effect of the processing conditions on the RS level. For example with the high amylose grain, increased steaming times led to a slight reduction in the level of RS, most likely due to increased starch gelatinization during steaming (Table 27). The wider roller gap generated a higher RS level except at the longest steaming time. This could have been due to increased shear damage of the starch granules when the grains were rolled at narrower gaps, reducing RS levels slightly. Narrower roller gaps also led to higher RS levels in the Hartog control, albeit at much lower overall RS levels. In contrast to the high amylose results, increased steaming times led to higher RS levels, possibly due to increased starch gelatinization at longer steaming times contributing to more starch retrogradation during subsequent processing and cooling.

Consolidated Data on RS from Various Products.

RS data obtained from various products such as noodles, sponge and dough bread and spaghetti, prepared as described in Example 10, are presented in Table 28. Not all levels of incorporation were tested for all products, but incorporation levels of 20%, 40% and 60% were used in most of the products analysed. The results showed a linear relationship between RS content and the level of incorporation of high amylose flour.

EXAMPLE 17

Isolation of Plants Having Point Mutations in SBEIIa

A population of mutated plant lines was developed after EMS mutagenesis of seeds of the wheat cultivars Arche or Apache, using standard EMS treatment conditions. About 5000 Apache and 900 Arche individual M1 plants were grown from the mutagenised seed, self-fertilised, and seeds from each plant and subsequent progeny generations maintained as potentially mutant lines, each derived from an individual M1 plant. The lines were screened for mutations in the three homoeologous SBEIIa genes by next-generation Solexa sequencing (Illumina). To do this, 7 DNA pools were prepared, each by pooling DNA from about 130 M1 families from the Arche population and 96 from the Apache population. PCR was carried out on the pooled DNAs for 3 or 4 regions per homoeologous gene, targeting the exonic regions including splice sites of the genes. Genome-specific primers are set out in Table 29.

The 10 amplicons (amplification products) from the same DNA pools were merged after normalization of the PCR products, and sequencing was done with one flow cell per DNA pool. The sequence data were analysed to select from all of the polymorphisms the ones most likely due to mutations rather than to sequencing errors, based on the frequencies of the observed polymorphisms. 64 putative mutants from the Arche population and 48 from the Apache population were observed from the first sequence analysis covering the exonic regions and splice sites. SNP assays were designed for each polymorphism based on kaspar technology, and genotyping was performed on the 130 families in each pool that was positive for the particular polymorphism. Thereby, the individual mutant line containing each mutant gene was identified and the mutant SBEIIa sequences confirmed.

By this method, 31 mutant lines from the Apache population and 9 from the Arche population were identified each having an SBEIIa mutation, and M2 kernels of each retained. From each mutant line, depending on availability, around 10 M2 seeds, were cut in half, the half without the embryo was used for DNA extraction and analysis, the other half with the embryo was saved for sowing. A total of 5 mutants were confirmed on half seeds from Arche population and 28 from Apache population. The corresponding seeds were sown to produce progeny plants to confirm that the mutations were inherited in Mendelian fashion by repeating analysis on M2 plant leaf material, providing much better DNA quality. These analyses confirmed 19 mutants, 4 from the Arche population and 15 from the Apache population and allowed their ranking depending on their DNA and the deduced protein sequences encoded by the mutants.

The obtained mutants included ones which had mutated SBEIIa genes with stop codons in the protein coding regions of the SBEIIa genes on the B or D genomes, causing premature termination of translation of the SBEIIa proteins, and lines with splice site mutations in the SBEIIa-B or -D genes. Such mutations were expected to be null mutations. Point mutations in the SBEIIa-A, SBEIIa-B and SBEIIa-D genes such as amino acid substitution mutations were also obtained and their impact on the structure of the encoded proteins predicted using Blosum 62 and Pam 250 matrices.

Plants from the most promising 8 mutant lines were crossed with double-null SBEIIa mutants of the appropriate genotype including A1D2, A2D2, A1B2, B2D2 genotypes in order to produce the triple-mutant plants and seed in the F2 generation. Fertile plants producing seeds with at least 50% amylose in the starch content are selected. Mutant plants were also crossed with durum wheat (cultivar Soldur) to introduce the mutations into the tetraploid wheat.

TABLE 1

Starch branching enzyme genes characterized from cereals

Species	SBE isoform	Type of clone	Accession No.	Reference
Maize	SBEI	cDNA	U17897	Fisher et al., *Journal Plant Physiol.* 108(3): 1313-1314, 1995
		genomic	AF072724	Kim et al., *Gene*. 216(2): 233-43, 1998a
	SBEIIb	cDNA	L08065	Fisher et al., *Plant Physiol.* 102: 1045-1046, 1993
		genomic	AF072725	Kim et al., *Plant Physiol.* 121(1): 225-236, 1999
	SBEIIa	cDNA	U65948	Gao et al., 1997
Wheat	SBEII	cDNA	Y11282	Nair et al., *Plant Sci* 122: 153-163, 1997
	SBEI	cDNA and genomic	AJ237897 (SBEI gene) AF002821 (SBEI pseudogene AF076680 (SBEI gene) AF076679 (SBEI cDNA)	Baga et al., *Plant Mol Biol*. 40(6): 1019-1030, 1999 Rahman et al., *Genome* 40: 465-474, 1997, Rahman et al., 1999
	SBEI	cDNA	Y12320	Repellin et al., *Plant Gene Reg* pp. 97-094, 1997
	SBEIIa	cDNA and genomic	AF338432 (cDNA) AF338431 (gene)	Rahman et al., 2001
	SBEIIa	cDNA	AK335707, AF286319	
	SBEIIb	cDNA and genomic		WO 01/62934
	SBEIIb	cDNA		WO 00/15810
	SBEIIb-D	cDNA		US2005074891
Rice	SBEI	cDNA	D10752	Nakamura, 2002 and Nakamura and Yamanouchi, *Plant Physiol*. 99(3): 1265-1266, 1992
	SBEI	genomic	D10838	Kawasaki et al., *Mol Gen Genet.* 237(1-2): 10-6, 1993
	RBE3	cDNA	D16201	Mizuno et al., 1993
Barley	SBEIIa and SBEIIb	cDNA and genomic	AF064563 (SBEIIb gene) AF064561 (SBEIIb cDNA) AF064562 (SBEIIa gene) AF064560 (SBEIIa cDNA)	Sun et al., 1998

TABLE 2

Amino acid sub-classification

Sub-classes	Amino acids
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine
Residues that influence chain orientation	Glycine and Proline

TABLE 3

Exemplary and Preferred Conserved Amino Acid Substitutions

Original Residue	Exemplary conservative substitutions	Preferred conservative substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe	Leu
Leu	Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr

TABLE 3-continued

Exemplary and Preferred Conserved Amino Acid Substitutions

Original Residue	Exemplary conservative substitutions	Preferred conservative substitutions
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala	Leu

TABLE 4

Genome specific primers for wheat SBEIIa genes

Genome	Primers	Region	Expected Size (bp)
A	SbeIIa_A_deb1F/SbeIIa_A_deb1R	Exons 1 to 8	615
A	SbeIIa_A_deb2F/SbeIIa_A_deb1R	Exons 1 to 8	604
A	SbeIIa_A_deb2F/SbeIIa_A_deb5R	Exons 1 to 8	~1039
A	SbeIIa_A_deb3F/SbeIIa_A_deb1R	Exons 1 to 8	565
A	SbeIIa_A_deb4F/AR2aE8R07	Exons 1 to 8	735
A	SbeIIa_A_deb5F/AR2aE8R07	Exons 1 to 8	696
B	SbeIIa_B_R4/BeIIaE1f	Exons 1 to 8	~600 on B, ~800 on A
D	SbeIIa_D_deb1F/SbeIIa_D_deb1R	Exons 1 to 8	573
D	SbeIIa_D_deb1F/SbeIIa_D_deb2R	Exons 1 to 8	539
D	SbeIIa_D_deb1F/SbeIIa_D_deb4R	Exons 1 to 8	~900
D	SbeIIa_D_deb2F/SbeIIa_D_deb4R	Exons 1 to 8	~900
D	SbeIIa_D_deb3F/SbeIIa_D_deb4R	Exons 1 to 8	~900
D	SbeIIa_D_deb4F/AR2aE8R07	Exons 1 to 8	736
A	Snp1for/Arev5	Exons 13-14	508
A	Afor4/del4rev	Exons 12-14	863
A	Snp6for/Arev5	Exon 14	205
A	Afor4/Snp6rev	Exons 12-13	637
A	Afor4/del5rev	Exons12-14	872
B	Bsnp4/Arev5	Exons13-14	494
B	Afor4/Bsnp17rev	Exons12-14	905
B	Afor4/Bsnp18rev	Exons 12-14	952
D	Afor4/Dsnp7rev	Exons 12-14	901
D	Dsnp7for/Drev1	—	278
D	Afor4/Arev5	Exons 12-14	802

TABLE 5

Nucleotide sequences of genome specific primers of SBEIIa

Primer name	Nucleotide Sequence (5' to 3')	SEQ ID NO:
SbeIIa_A_deb1F	GTTCGATGCTGTTCCCCAG	36
SbeIIa_A_deb1R	AGCCGTTTGCTCCTCGATG	37

TABLE 5 -continued

Nucleotide sequences of genome specific primers of SBEIIa

Primer name	Nucleotide Sequence (5' to 3')	SEQ ID NO:
SbeIIa_A_deb2F	TTCCCCAGTTGATCTCCATC	38
SbeIIa_A_deb4F	CTTACTGAATACTGACCAGTTG	39
SbeIIa_A_deb5F	TTTATGATCTGGCTTTTGCATCCTA	40
SbeIIa_A_deb5R	GATGTTCCCCAAATTTGCATGAC	41
SbeIIa_B_deb4R	AATGCACAAGGCAGTGAAGTAG	42
SbeIIa_D_deb1F	CCCAATTGATCTCCATGAGT	43
SbeIIa_D_deb1R	AACCCCAAACGGTGCATTATG	44
SbeIIa_D_deb2F	CGGCTTTGATCATTCCTCG	45
SbeIIa_D_deb2R	GCTAGAATGCACATCCATCTGAT	46
SbeIIa_D_deb3F	GTAACTGCAAGTTGTGGCG	47
SbeIIa_D_deb4F	GCTTACTGAATACTGACCAGTTACTA	48
SbeIIa_D_deb4R	CCTTAATTCAAAATGAGCGAAAGC	49
snp1for	GGCTAACTGTTCCTGTTAAA	50
snp6for	GATGAGATCATGGACGATTC	51
snp6rev	AATAAATAATAATCACTTCG	52
Del4rev	GAGTAACAGCCTGATCCCAA	53
Del5rev	TAACAAAAAGAGTAACAGCC	54
Bsnp4	GTCAATCTGTTCTTACACG	55
Bsnp17 rev	CAAAAAGAGTAGTAACAGCT	56
Bsnp18 rev	CAAGGTATAAATTAGCATTC	57
D snp7 for	GTTTTATTTTGGGGATCAGT	58
D snp7 rev	CCCTAACAAAAAGTGTAACAGA	59
Afor4	ATCAGACCTTGTCACCAAAT	60
Arev5	GCACTTACATCTTCACCAATG	61
Drev 1	GCCTTCTGAAGCAATTGACAAG	62

TABLE 6

Primers designed to amplify parts of the SBEIIa gene specifically from the A genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Afor4	Arev5	Arev6	SEQ ID NO:
snp1for	GGCTAACTGTTCCTGTTAAA	extra A/B and D		508		63
SNP1REV	CGACATGTGTAAGAACAGAT	extra A/B and D	334			64
snp2for2a	GTCGATATTCTATTCTTATGT	t/D; a/B; a/B; c/B D		474		65
snp3for	CTTTTTTAGGGCACTGAAAT	c/B; dB; c/B D		315		66
snp3reva	GTTATGATGCATAGCAATTA	c/B D	528			67
snp4for	TCTTAGATAGTTCCCTAGTAC	t/B D		245		68
snp4rev	CAGGTAAAATTGTACAAGCG	t/B D	599			69

TABLE 6 -continued

Primers designed to amplify parts of the SBEIIa gene specifically from the A genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Afor4	Arev5	Arev6	SEQ ID NO:
snp5for	ACCTGATGAGATCATGGAC	a/B D		210		70
snp5for2	TACCTGATGAGATCATGGAC	a/B D		211		71
snp6for	GATGAGATCATGGACGATTC	a/B D; g/B D		205		72
snp6rev	AATAAATAATAATCACTTCG	t/B; a/B; g/B; g/B D	637			73
snp7for	TCTTTTTGTTAGGGGTAAG	3 first bp extra/D; extra act in BD; a/B D			390	74
A for3	AGTTTGACCAAGTCTACTG			1050		75
Afor4	ATCAGACCTTGTCACCAAAT	t/D		802		76
Arev5	GCACTTACATCTTCACCAATG		802			77
Arev7	GTAGTTATAAGCAATATG					78
del1for	CATCAAGTGGTTTCAGTAAC	7 by Difference/BD		334		79
del1rev	GTTACTGAAACCACTTGATG		490			80
Del4for	TTGGGATCAGGCTGTTACTC	extra g in B D; t = a BD; extra act in BD				81
Del4rev	GAGTAACAGCCTGATCCCAA		863			82
Del5for	GGCTGTTACTCTTTTTGTTA	t/BD; extra t; act extra in BD; extra ct				83
Del5rev	TAACAAAAAGAGTAACAGCC		872			84
Del3for	TTAACCAGTTAAGTAGTT	extra cagt; extra a; extra ttaag in D and ttaatag in B			432	85
Del3rev1	AACTACTTAACTGGTTAA	extra ttaag in D and ttaatag in B; extra a; extra actg	836			86
Del3rev2	GATCCCAAAATAAAACTACTT	extra ttaag in D and ttaatag in B; extra a	851			87
Del3rev3	CCCAAAATAAAACTACTT	extra ttaag in D and ttaatag in B; extra a	848			88

TABLE 7

Primers designed to amplify parts of the SBEIIa gene specifically from the B genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Arev5	Afor4	Exons	SEQ ID NO;
Bsnp1for	GTGGGATTCTCGTCTG	a/A D				89
Bsnp2	TTGGGAAGTATGTAGCTGC	ct/A D	546		13_14	90
Bsnp3	TTGGCTAACTGTTCCTGTC	t/A D	509		13_14	91
Bsnp4	GTCAATCTGTTCTTACACG	t/A D; extra a in A; a/A D	494			92
Bsnp5	ATCTGTTCTTACACGTGTCA	a/A D; t/D; g/A D	494			93
Bsnp6	GTCAATATTCTATTCTTATA	t/D; g/A D; g/A D	474			94
Bsnp7	CTATTCTTATACAGGTATTA	g/A D; g/A D	465			95
Bsnp8	AACGCGAGATGGTGGCTTGAT	a/A D	430		half 13_14	96
Bsnp9	CAAGTGGTTTCAGTAACTTC	t/A D	331		14	97
Bsnp10	TGGTTTCAGTAACTTCTTC	t/A D; t/A D	327			98

TABLE 7 -continued

Primers designed to amplify parts of the SBEIIa gene specifically from the B genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Arev5	Afor4	Exons	SEQ ID NO;
Bsnp11	GGAAGATTGGAAGTGATTG	c/A; c/A; a/A D	195		14	99
Bsnp13	TGGAAGTGATTGTTATTAT	a/A D; ta/A D	188			100
Bsnp14	TTGCTTCTTGTTCTAGATGG	t/D; a/A D	155			101
Bsnp1rev	TTCCCAACTCCCATAGTGAAC	a/A D		290	half 12	102
Bsnp2rev	CAAATATGGTGACAGAAGTCG	tc/A D		322		103
Bsnp3rev	CACGTGTAAGAACAGATTG	a/A D; extra a in A; t/A D		356		104
Bsnp4rev	AGAATAGAATATTGACAC	g/A D; t/D; g/A D		371		105
Bsnp6rev	GTAAGAATCTTAATACCTGT	g/A D; g/A D		396		106
B snprev7	CGCGTTTGACAGTAAGAATCTT	g/A D		405	13	107
Bsnp8rev	CCATCAAACTTATATTCA	a/A D		437		108
Bsnp9rev	CAATTGTTTCAGTGCCCTGAAG	t/A; t/A D; t/A D		539	12_13	109
Bsnp10rev	GCAATTGTTTCAGTGCCCTG	t/A; t/A D		540		110
Bsnp11rev	CTTAGAAGAAAAAATAATAAC	c/D; ta/A D; a/A D		673	12_13	111
Bsnp13rev	GCAAACTTAGAAGAAAAAA	t/D; c/D; a/A D		678		112
Bsnp14rev	CCATAGTTCCCAGTAAATGC	a/A D		713	12_13	113
Bextra1rev	CTACTATTAAATTAACTG	ct extra/A, at extra/AD, taa extra/A, g/AD, actg extra/D		868	12_14	114
Bsnp16 rev	ATCCCCAAAATAAAACTACTAT	c extra/A, tat extra /AB		880	12_14	115
Bsnp17 rev	CAAAAAGAGTAGTAACAGCT	ag extra/D, agt extra/A, a extra/D, t/D, g/AD		905	12_14	116
Bsnp18 rev	CAAGGTATAAATTAGCATTC	c/AD		952	12_14	117
Bsnp19 rev	GCATTCTTATGAAAAGAC	c/AD, c/AD		938	12_14	118

TABLE 8

Primers designed to amplify parts of the SBEIIa gene specifically from the D genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Arev5	Drev 1	Afor4	SEQ ID NO:
D snp1for	TCTGTFCTTACACATGTT	c/A B	489	798		119
D snp1for/A	CTTTTTTAGGGCACTGAAAC	c/B; c/B; t/A	315	624		120
Dsnp2 for	GATTATTATTTATTTTTCCTTCTAAGTTTGT	g/B; at/B; t/AB; cAB	184	490		121
Dsnp2bfor	ACCTGATGAGATCATGGAAGATTG	c/A; c/A	210	519		122
D sap 3 for	GTGATTATTATTTATTTTC	g/B; at/B; t/AB; cAB	183	492		123
D sup 4 for	TTATTTTCCTTCTAAGTTTGT	at/B; t/AB; c/AB	172	481		124
D snp5for	GTGATTATTATTTATTTTC	g/B; at/B; t/AB	137	446		125
D snp6for	TGATGCGGTAGTTTACTTGATGT	g/B; a/B; c/AB	89	398		126
D del1for	GATTTTTAACTAGTTAAGTAGTT	t/B; cagt/AB; a/AB; t/B; at/B; del in A		298		127

TABLE 8 -continued

Primers designed to amplify parts of the SBEIIa gene specifically from the D genome of wheat-detected polymorphisms and fragment sizes

Primer code	Primer sequence	SNP details	Arev5	Drev 1	Afor4	SEQ ID NO:
D snp7 for	GTTTTATTTTGGGGATCAGT	del g in A; a/B; g/AB		278		128
D snp1 rev	CCTGCATAAGAATAGAATATCA	t/A; a/B; c/AB			379	129
D snp1a rev	CATGTTATGATGCATAGCAATTG	t/A			556	130
D snp2 rev	GTAAATGTCATCTAGAACAAGAAA	g/B; c/AB			701	131
D snp3 rev	CAAGAAACAAACTTAGAAGG	c/AB; t/AB			684	132
D snp4 rev	ACAAACTTAGAAGGAAAATAA	c/AB; t/AB; at/B			678	133
D snp5 rev	CATCAGTAGCAAATCCAAAATAT	g/AB			739	134

TABLE 9

Genome specific primers for wheat SBEIIb genes

Genome	Primers	Expected Size (bp)
A	SbeIIb_A_deb1F/2R	741
A	SbeIIb_A_deb1F/4R	1007
A	SbeIIb_A_deb4F/4R	772
B	SbeIIb_B_deb3F/2R	615
B	SbeIIb_B_deb2F/3R	929
B	SbeIIb_B_deb3F/4R	772
D	SbeIIb_D_deb1F/1R	1126
D	SbeIIb_D_deb3F/3R	827
D	SbeIIb_D_deb4F/4R	669

TABLE 11

SBEII expression vs Amylose content of RNAi lines of wheat

selected line	Construct	Amylose %	SBEIIa expression relative to a WT (%)	SBEIIb expression relative to a WT (%)	Total SBEII expression (% of WT)
673.2.1	hp-combo	35	108	91	100
679.5.3	hp-combo	40	81	1	41
670.1.4	hp-combo	45	35	10	23
672.2.3	hp-combo	50	16	1	9
671.2.2	hp-combo	55	8	5	7
666.2.2	hp-combo	60	10	6	8
669.1.2	hp-combo	65	9	7	8

TABLE 10

Nucleotide sequences of genome specific primers of SBEIIb

Primer name	Nucleotide Sequence (5' to 3')	SEQ ID NO:
SbeIIb_A_deb1F	ACCCCGTAATTATTGGCGCT	135
SbeIIb_A_deb4F	ACTCTGATGATCTGAAGGTAG	136
SbeIIb_A_deb2R	TCATGCAGGCAGGTACTAG	137
SbeIIb_A_deb4R	GTGGCAGAATGCGTAATTTCTCT	138
SbeIIb_B_deb2F	CAGCGATCTTACGTTCCCTA	139
SbeIIb_B_deb3F	ATGTCTGTAGGTGCCGTCA	140
SbeIIb_B_deb2R	CAACAAATTAGAAAGAGGATATTCC	141
SbeIIb_B_deb3R	CCGTAGATGATTCTTTGTCCATTA	142
SbeIIb_B_deb4R	ATGGAACCTAACACAATGTGC	143
SbeIIb_D_deb1F	GCGCCACCTTTCTCACTCA	144
SbeIIb_D_deb3F	CGGTCCCGTTCAGTTCGAT	145
SbeIIb_D_deb4F	CCTGAGTAAATACTGCCACCA	146
SbeIIb_D_deb1R	AGAATGCGTAATTTCTCCCTCG	147
SbeIIb_D_deb3R	TGTCTTCAGCATCAATTTCTTCAC	148
SbeIIb_D_deb4R	CTGTAGGCTTGTTTCATCATCA	149

TABLE 11-continued

SBEII expression vs Amylose content of RNAi lines of wheat

selected line	Construct	Amylose %	SBEIIa expression relative to a WT (%)	SBEIIb expression relative to a WT (%)	Total SBEII expression (% of WT)
684.2.3	hpc-SBEIIa	70	6	10	8
677.1.2	hp-combo	75	4	1	3
684.2.1	hpc-SBEIIa	80	3	5	4
694.3.3	hpc-SBEIIa	85	2	3	3

TABLE 12

List of microsatellite markers tested in the mutants

Chromosome 2A	Chromosome 2B	Chromosome 2D
gwm 304	barc 128	gwm 539
gwm 328	gwm 129	cfd 270
barc 309	wmc 265	cfd 168
cfa 2043	wmc 272	cfd 233
cfa 2058	gwm 388	wmc 175
wmc 170	wmc 441	wmc 181
gwm 312	barc 101	wmc 041
gwm 294	gwm 120	cfd 239
wmc 181	gwm 130	gwm 349
gwm 356	gwm 526	barc 219
gwm 265	gwm 501	gwm 382
wmc 181	wmc 332	wmc 167
gwm 311	wmc 434	gwm 320
gwm 382	wmc 361	gwm 301
cfa 2086	gwm 382	cfd 50
	wmc 317	barc 159
	wmc 445	

TABLE 13

Mutants identified from HIB population and microsatellite mapping data

Mutant type	Genome	Mutant number	Microsatellite mapping (markers retained/ markers tested)
Type 1	A	20-257 (H7)	15/15
		19-119 (G3)	5/11
		12-178	10/10
		5-563	10/10
		21C-880D	4/10
	B	12-679	7/15
		5-173	15/15
		13-963 (F10)	4/11
		18c-109	8/8
		3-159	3/8
	D	19-832 (A6)	13/13
		22-578 (B5)	13/13
		3-909 (D1)	7/13
		19b-918 (C11)	To be done
Type 2	A	20b-5B2-608 (H2)	10/10
		19c-342	9/10
		19-744	12/12
	B	21-142 (F6),	15/15
		21-668 (D2-2)	15/15
		20-365	15/15
		19-220	14/15
		21b-4B2-345 (A8)	11/11
		20-141	9/11
	D	12-801	13/13
		5-706	13/13
		19c-905	To be done
		18b-505	To be done
Type 3	A	18-111/3 (D2-1)	8/11
		19-861 (F9)	8/11
		20-791 (G10)	12/12
	B	19b-55 (G7)	11/11

TABLE 13-continued

Mutants identified from HIB population and microsatellite mapping data

Mutant type	Genome	Mutant number	Microsatellite mapping (markers retained/ markers tested)
	D	18-96 (E12)	18/18
		18b-120 (E3)	To be done
		18b-190 (C12)	To be done

TABLE 14

Double null mutants of SBEII identified

Cross designation	Parental lines (genotype of parent)	Genotype of double null	Number of double nulls identified
08/a	20-257 (A1) × 5-173 (B1)	A1B1	6
08/b	20-257(A1) × 19-832 (D1)	A1D1	2
08/c	19-832 (D1) × 5-173 (B1)	B1D1	0
08/d	21-142 (B2) × 12-801 (D2)	B2D2	4
08/e	21-142 (B2) × 5-706 (D2)	B2D2	8
08/f	20-365 (B2) × 12-801(D2)	B2D2	4
08/g	21-668 (B2) × 5-706 (D2)	B2D2	6
08/h	20-257 (A1) × 21-142 (B2)	A1B2	2
08/i	20-257 (A1) × 12-801 (D2)	A1D2	5
08/j	18-111/3 (A3) × 18-96 (D3)	A3D3	2
08/k	18-111/3 (A3) × 5-173 (B1)	A3B1	3
08/l	18-96 (D3) × 5-173 (B1)	B1D3	1

TABLE 15

Crosses performed between double and single null mutants

Cross designation	Parent 1 Code	P1 genotype	Parent 2 Code	P2 genotype	Potential F2 genotype
08/aa	5-173	B1	08/b-18	A1D1	A1B1D1
08/aa-2	5-173	B1	08/b-33	A1D1	A1B1D1
08/bb	5-706	D2	08/h-92	A1B2	A1B2 D2
08/dd	5-706	D2	08/h-111	A1B2	A1B2 D2
08/ee	5-173	B1	08/b-12	A1D1	A1B1D1
08/ff	21-142	B2	08/b-12	A1D1	A1B2D1
08/gg	20-365	B2	08/b-12	A1D1	A1B2D1

TABLE 16

Amylose content in grain starch of progeny from crosses between double null mutants and single null mutants

Lines	Genotype	Amylose %
HIB mutant	F2 of triple null cross	67.38
Cadoux	WT	35.4
85.2c	hp-SBEIIa	74.99
008 (IIb knock out)	hp-SBEIIb	36.1
Chara	WT	36.09

TABLE 17

Fertility observations on F2 progeny plants

Line ID	Genotype	% fertile spikes	Number of seed per head
08/dd S28	A1D2(hetB2)	41.9	17.0
08/dd S14	A1B2(hetD2)	75.3	26.3
08/dd S22	A1D2(hetB2)	56.5	19.0
08/dd S24	B2D2(hetA2)	61.1	16.0

TABLE 17-continued

Fertility observations on F2 progeny plants

Line ID	Genotype	% fertile spikes	Number of seed per head
08/dd-2 D7	A1B2	84.2	37.3
08/dd-2 F1	B2	93.2	50.7
08/dd-2 G7	A1D2	92.6	49.7
08/dd-2 A1	B2D2	91.5	44.3

TABLE 17-continued

Fertility observations on F2 progeny plants

Line ID	Genotype	% fertile spikes	Number of seed per head
08/dd-2 F4	D2	84.4	45.7
08/dd-2 D5	wt	95.3	49.0

TABLE 18

SBEII allelic composition of mutants with multiple SBEIIa and SBEIIb null alleles

Plant	Number of wild-type SBEIIa alleles present on A, B and D genomes						Total number of wild-type SBEIIa	Number of wild-type SBEIIb alleles present on A, B and D genomes						Total number of wild-type SBEIIb	Total number of wild-type SBEIIa and SBEIIb alleles	Amylose
Genotype	A	A	B	B	D	D	alleles present	A	A	B	B	D	D	alleles present	present	content %
A1(+/−)B2D2	1	—	—	—	—	—	1/6	1	—	1	1	1	1	5/6	6/12	67% (pooled)
A1B2D2(+/−)	—	—	—	—	1	—	1/6	—	—	1	1	1	1	4/6	5/12	67% (pooled)
A1B2(+/−)D2	—	—	1	—	—	—	1/6	—	—	1	1	1	1	4/6	5/12	67% (pooled)
B2D2	1	1	—	—	—	—	2/6	1	1	1	1	1	1	6/6	8/12	33.0-36.8
A1B2	—	—	—	—	1	1	2/6	—	—	1	1	1	1	4/6	6/12	33.9-34.9
A1D2	—	—	1	1	—	—	2/6	—	—	1	1	1	1	4/6	6/12	32.2-37.0
A1B1	—	—	—	—	1	1	2/6	—	—	—	—	1	1	2/6	4/12	34.1-34.7
A1D1	—	—	1	1	—	—	2/6	—	—	1	1	—	—	2/6	4/12	32.8-38.7
A3D3	1	1	1	1	1	1	6/6	—	—	1	1	—	—	2/6	8/12	30.8-31.6
A3B1	1	1	—	—	1	1	4/6	—	—	—	—	1	1	2/6	6/12	31.4
B1D3	1	1	—	—	1	1	4/6	1	1	—	—	—	—	2/6	6/12	30.3

TABLE 19

Further crosses between single and double null mutants

Cross	Parent 1 Code	P1	Parent 2 Code	P2	Potential Triple Mutant genotype	Observed progeny genotypes
08/hh-1	5-173	B1	08/i-G3	A1D2	A1B1D2	All possible single nulls and A1B2 double nulls identified, No triple nulls
08/ii-1	20-365	B2	08/i-G3	A1D2	A1B2D2	All possible single nulls and B2D2 double null identified, No triple nulls
08/ii-2	20-365	B2	08/i-C1	A1D2	A1B2D2	All possible single nulls, B2D2 and A1D2 double nulls identified, no triple nulls
08/ii-3	20-365	B2	08/i-C8	A1D2	A1B2D2	All three double nulls identified, but no triple nulls.
08/hh-4	5-173	B1	08/i-B12	A1D2	A1B1D2	All three double nulls identified, but no triple nulls.
08/kk-2	5-563	A1	08/g-8G	B2D2	A1B2D2	All possible single nulls and double nulls identified, no triple nulls
08/kk-3	5-563	A1	08/g-A1	B2D2	A1B2D2	All possible single nulls and A1D2 and A1B2 double nulls identified, no triple nulls
08/kk-4	5-563	A1	08/g-D8	B2D2	A1B2D2	All single and double nulls identified, No triple nulls

TABLE 19-continued

Further crosses between single and double null mutants

Cross	Parent 1 Code	P1	Parent 2 Code	P2	Potential Triple Mutant genotype	Observed progeny genotypes
08/kk-6	5-563	A1	08/g-E10	B2D2	A1B2D2	All possible single nulls and only B2D2 double nulls identified
08/ll-1	20-257	A1	08/g-E7	B2D2	A1B2D2	All possible single nulls and A1B2 and B2D2 identified, no triple nulls
08/ll-2	20-257	A1	08/g-8G	B2D2	A1B2D2	All possible single nulls and double nulls identified, no triple nulls
08/ll-4	20-257	A1	08/g-D8	B2D2	A1B2D2	All possible single nulls and A1D2 and B2D2 identified, no triple nulls
08/ll-6	20-257	A1	08/g-E10	B2D2	A1B2D2	All possible single nulls and double nulls identified. No triple nulls
08/mm-1	19c-342	A2	08/d-C7	B2D2	A2B2D2	All possible single and double nulls identified. No triple nulls
08/mm-2	19-744	A2	08/f-C8	B2D2	A2B2D2	No triple null
08/mm-3	19-744	A2	08/f-G9	B2D2	A2B2D2	No triple null
08/mm-4	19-744	A2	08/e-F5	B2D2	A2B2D2	No triple null
08/mm-5	19-744	A2	08/e-C11	B2D2	A2B2D2	No triple null
08/mm-6	19-744	A2	08/d-E8	B2D2	A2B2D2	No triple null
08/mm-7	19-744	A2	08/d-D11	B2D2	A2B2D2	No triple null
08/mm-8	19-342	A2	08/f-C8	B2D2	A2B2D2	No triple null
08/mm-9	19-342	A2	08/f-C8	B2D2	A2B2D2	No triple null
08/mm-10	19-342	A2	08/f-C8	B2D2	A2B2D2	No triple null
08/mm-11	19-342	A2	08/f-C8	B2D2	A2B2D2	No triple null
08/mm-12	19-342	A2	08/f-C8	B2D2	A2B2D2	No triple null

TABLE 20

Observed frequency of genotypes of normally germinating grain from an A2B2D2 cross.

	WT	A2	B2	D2	A2B2	A2D2	B2D2	A2B2D2
08/mm1-4	69	7	9	4	2	3	2	0
08/mm1-6	56	16	10	9	2	1	2	0
08/mm1-7	44	18	19	9	1	3	2	0
08mm1-5	54	13	15	10	1	1	2	0
08mm1-2	56	12	12	10	4	0	2	0
08/mm1-3	53	12	13	14	1	1	2	0
08/mm1-1	54	13	13	11	1	3	1	0
Total observed (expected)	386 (283)	90 (95)	91 (95)	67 (95)	12 (32)	12 (32)	13 (32)	0

Numbers in parentheses indicate the expected frequency based on Mendelian segregation

TABLE 21

Further crosses between single and double null mutants

Cross desig-nation	Parent 1	Parent 2	Triple null genotype sought	Screening status-(number screened)
09/aa-1	08/k-F9 (18-111/3 × 5-173) A3B1	19-832 (D1)	A3B1D1	285 (only A3B1 double null recovered)

TABLE 21-continued

Further crosses between single and double null mutants

Cross desig-nation	Parent 1	Parent 2	Triple null genotype sought	Screening status-(number screened)
09/bb-1	08/d-E8 (21-142 × 12-801) B2D2	18-111/3 (A3)	A3B2D2	285 (2 confirmed A3B2D2 triple nulls).

TABLE 21-continued

Further crosses between single and double null mutants

Cross desig-nation	Parent 1	Parent 2	Triple null genotype sought	Screening status-(number screened)
09/cc-1	08/b-12 (20-257 × 19-832) A1D1	19b-55 (B3)	A1B3D1	285 (Only B3D1 double null recovered)
09/dd-1	08/i (20-257 × 12-801) A1D2	19b-55 (B3)	A1B3D2	190 (A1D2 and A1B3 double nulls recovered)
09/ee-1	08/1-G9 (18-96 × 5-173) B1D3	20-257 (A1)	A1B1D3	190 (No double nulls recovered)
09/ff-1	08/1-G9 (18-96 × 5-173) B1D3	19c-342 (A2)	A2B1D3	190/190 (Results not clear)
09/gg-1	08/j-D4 (18-111/3 × 18-96) A3D3	19b-55 (B3)	A3B3D3	190 (1 confirmed triple null)

TABLE 22-continued

Putative double and triple null mutants in SBEIIa genes identified in an initial screen using dominant markers

Cross ID Number	Cross combination	Putative double mutants	Putative triple mutants
M82	Type I-20.257 (A)/APACHE// Type II-12.801 (D)/Type II-21.142 (B)	128	4
M83	Type I-20.257 (A)/APACHE// Type II-12.801 (D)/Type II-21.668 (B)	83	4
M84	Type II-5.706 (D)/Type II-21.668 (B)//Type I-20.257 (A)/APACHE	36	1
M85	Type II-5.706 (D)/Type II-21.668 (B)//Type I-20.257 (A)/CHARA	171	8
M86	Type II-12.801 (D)/Type II-21.142 (B)//Type I-20.257 (A)/CHARA	69	1
M78	Type II-21.142(B)/Type I-20257 (A) [08/h-92]//Type I-19.832 (D)/CHARA	18	0
M80	Type II-21.142(B)/Type I-20257 (A) [08/h-111]//Type I-19.832 (D)/CHARA	31	1
		793	21

TABLE 23

Starch characterisation of grain starch from transgenic wheat lines

Line ID	Enzyme targeted	Birefringence nil (%)	Birefringence partial (%)	Birefringence Full (%)	Amylose content estimated iodometrically (%)	Amylose content determined by SEC %	Starch content (% w/w)	Starch swelling power
NB1	Non-transformed	1.6	3.5	94.9	31.8	25.5	52.0	9.31
SBEIIa-1	SBEIIa	94.5	4.0	1.5	88.5	74.4	43.4	3.51
SBEIIb-1	SBEIIb	0.6	5.21	94.1	27.3	32.8	50.3	10.74
LSD (5%)		9.02	3.3	9.9	7.7	nd	4.9	

TABLE 22

Putative double and triple null mutants in SBEIIa genes identified in an initial screen using dominant markers

Cross ID Number	Cross combination	Putative double mutants	Putative triple mutants
M76	Type I-19.832 (D)/APACHE// Type II-21.142(B)/ Type I-20257(A) [08/h-92]	107	2
M77	Type I-19.832 (D)/APACHE// Type II-21.142(B)/ Type I-20257(A) [08/h-111]	46	0
M79	Type II-21.142(B)/Type I-20257 (A) [08/h-111]//Type I-22.578 (D)/APACHE	73	0
M81	Type II-21.142(B)/Type I-20257 (A) [08/h-111]//Type I-22.578 (D)/CHARA	14	0
M74	Type I-5.173 (B)//Type I-19.832 (D)/Type II-20.257(A) [08/b12]	16	0
M75	Type I-19.832 (D)/Type II-20.257(A) [08/b12]//Type I-3.159 (B)	1	0

TABLE 24

Molecular weight distribution of starch fractions from wheat transgenic lines

Line	Estimated Molecular Weight (kDa) Amylopectin	High MW amylose	Low MW amylose
Wild-type (control)	45523.3 ± 2605.3	420.4 ± 23.2	8.56 ± 0.2
Reduced for SBEIIb	43646.4 ± 5259.6	409.6 ± 7.8	8.76 ± 0.1
Reduced for SBEIIa and SBEIIb	7166.1 ± 166.5	422.7 ± 26.8	9.70 ± 0.1

TABLE 25

RVA parameters of hp5'-SBEIIa transgenic wheat starch

Line ID	Construct	Peak 1	First Trough	Break-down	Final Viscosity	Setback	Peak Time	Pasting Temp (° C.)
Control	none	225.08	180.83	44.25	318	137.17	10	85.3
SBEIIa	hp5'-BEIIa	27.08	17.5	9.58	22.92	5.42	12.73	*

* Starch from the reduced SBEIIa grain (line 85.2c) did not paste at the temperature profile used in the RVA run.

TABLE 26

DSC parameters of gelatinisation peak of hp5'-SBEIIa transgenic wheat starch compared to the control NB1

Line ID	Construct	Onset ° C.	Peak ° C.	End ° C.	Delta H
NB1	Control	57.93	61.16	66.61	5.036
85.2c	hp5'-SBEIIa	57.38	63.51	72.61	2.385

TABLE 27

RS content in rolled and flaked grain products

Treatment No	Line	Roller width	Steaming time (Minute)	% RS (g/100 g product)
HWFP03	HAW	Wide	60	13.3
HWFP05	HAW	Wide	45	14.1
HWFP08	HAW	Narrow	35	13.7
HWFP09	HAW	Wide	35	16.1
HWFP11	HAW	Narrow	60	13.1
HWFP12	HAW	Narrow	45	11.4
HWFP01	Hartog	Narrow	60	0.6
HWFP02	Hartog	Wide	60	0.6
HWFP04	Hartog	Wide	45	0.5
HWFP06	Hartog	Narrow	45	0.4
HWFP07	Hartog	Narrow	35	0.1
HWFP10	Hartog	Wide	35	0.2

TABLE 28

Resistant starch content in food products at varying level of incorporation of high amylose wheat (HAW)

	Resistant Starch (g/100 g product) Incorporation level											
	0%		20%		40%		60%		80%		100%	
Type of product	control	HAW	Control	HAW	Control	HAW	Control	HAW	Control	HAW	Control	HAW
S & D bread	NT	NT	0.45	1.33	0.40	2.1	0.30	2.9	NT	NT	NT	NT
YAN	0.4	0	0.2	0.7	0	1.1	0.2	1.2				
Spaghetti			0.3	1.3	0.1	2	0	2.9	0.1	4	0	6
Instant noodle	0.4	0.4	0.3	0.8	0.2	1.4	0.2	1.6	NT	NT	NT	NT
Loaf bread	NT	NT	0.6	1.7	NT	NT	0.6	3.7	NT	NT	1	5.2
Flakes	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.2	16.1

NT: Not tested

TABLE 29

Genome-specific primers

SbeIIa SeqId	Primer pair	Covered exons
IIaA2_3	SbeIIa_A_deb2F/SbeIIa_A_deb5R	2, 3
IIaA6_7_8	SbeIIa_A_deb4F/AR2aE8R07	6, 7, 8
IIaA12_14	Del5rev/Afor4	12, 14
IIaB2_3	SbeIIa_Bdeb7F/BeIIaE3r	2, 3
IIaB12_14	BSNP17rev/Afor4	12, 14
IIaB21_22	Sbe2a_Bfin-F2/BeIIaE22r	21, 22

TABLE 29-continued

Genome-specific primers

SbeIIa SeqId	Primer pair	Covered exons
IIaD2_3	SbeIIa_D_deb1F/SbeIIa_D_deb4R	2, 3
IIaD6_7_8	SbeIIa_D_deb4F/AR2aE8R07	6, 7, 8
IIaD12_14	DSNP7rev/Afor4	12, 14
IIaD18_20	Sbe2a_Dfin-F1/Sbe2a_Dfin-R3	18, 20

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 154

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<211> LENGTH: 823
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 1

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Met Ala Thr Phe Ala Val Ser Gly Ala Thr Leu Gly Val Ala Arg Pro
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Ala Gly Ala Gly Gly Gly Leu Leu Pro Arg Ser Gly Ser Glu Arg Arg
            20                  25                  30

Gly Gly Val Asp Leu Pro Ser Leu Leu Leu Arg Lys Lys Asp Ser Ser
        35                  40                  45

Arg Ala Val Leu Ser Arg Ala Ala Ser Pro Gly Lys Val Leu Val Pro
    50                  55                  60

Asp Gly Glu Ser Asp Asp Leu Ala Ser Pro Ala Gln Pro Glu Glu Leu
65                  70                  75                  80

Gln Ile Pro Glu Asp Ile Glu Glu Gln Thr Ala Glu Val Asn Met Thr
                85                  90                  95

Gly Gly Thr Ala Glu Lys Leu Glu Ser Ser Glu Pro Thr Gln Gly Ile
            100                 105                 110

Val Glu Thr Ile Thr Asp Gly Val Thr Lys Gly Val Lys Glu Leu Val
        115                 120                 125

Val Gly Glu Lys Pro Arg Val Val Pro Lys Pro Gly Asp Gly Gln Lys
    130                 135                 140

Ile Tyr Glu Ile Asp Pro Thr Leu Lys Asp Phe Arg Ser His Leu Asp
145                 150                 155                 160

Tyr Arg Tyr Ser Glu Tyr Arg Arg Ile Arg Ala Ala Ile Asp Gln His
                165                 170                 175

Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys Leu Gly Phe
            180                 185                 190

Thr Arg Ser Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala
        195                 200                 205

His Ser Ala Ala Leu Val Gly Asp Phe Asn Asn Trp Asn Pro Asn Ala
    210                 215                 220

Asp Thr Met Thr Arg Asp Asp Tyr Gly Val Trp Glu Ile Phe Leu Pro
225                 230                 235                 240

Asn Asn Ala Asp Gly Ser Pro Ala Ile Pro His Gly Ser Arg Val Lys
                245                 250                 255

Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile Ser Ala Trp
            260                 265                 270

Ile Lys Phe Ser Val Gln Ala Pro Gly Glu Ile Pro Phe Asn Gly Ile
        275                 280                 285

Tyr Tyr Asp Pro Pro Glu Glu Glu Lys Tyr Val Phe Gln His Pro Gln
    290                 295                 300

Pro Lys Arg Pro Glu Ser Leu Arg Ile Tyr Glu Ser His Ile Gly Met
305                 310                 315                 320

Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Ala Asn Phe Arg Asp Glu
                325                 330                 335

Val Leu Pro Arg Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met
            340                 345                 350

Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr
        355                 360                 365
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Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Glu Asp Leu Lys
    370                 375                 380

Ser Leu Ile Asp Arg Ala His Glu Leu Gly Leu Leu Val Leu Met Asp
385                 390                 395                 400

Ile Val His Ser His Ser Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly
                405                 410                 415

Phe Asp Gly Thr Asp Thr His Tyr Phe His Gly Gly Pro Arg Gly His
            420                 425                 430

His Trp Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Ser Trp Glu Val
        435                 440                 445

Leu Arg Phe Leu Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys
    450                 455                 460

Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met Tyr Thr His
465                 470                 475                 480

His Gly Leu Gln Met Thr Phe Thr Gly Asn Tyr Gly Glu Tyr Phe Gly
                485                 490                 495

Phe Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Val Asn Asp
            500                 505                 510

Leu Ile His Gly Leu His Pro Asp Ala Val Ser Ile Gly Glu Asp Val
        515                 520                 525

Ser Gly Met Pro Thr Phe Cys Ile Pro Val Pro Asp Gly Gly Val Gly
    530                 535                 540

Leu Asp Tyr Arg Leu His Met Ala Val Ala Asp Lys Trp Ile Glu Leu
545                 550                 555                 560

Leu Lys Gln Ser Asp Glu Ser Trp Lys Met Gly Asp Ile Val His Thr
                565                 570                 575

Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser
            580                 585                 590

His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met
        595                 600                 605

Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser Thr Pro
    610                 615                 620

Arg Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Val Thr
625                 630                 635                 640

Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe
                645                 650                 655

Gly His Pro Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Thr Leu Pro
            660                 665                 670

Thr Gly Lys Val Leu Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg
        675                 680                 685

Arg Arg Phe Asp Leu Gly Asp Ala Asp Phe Leu Arg Tyr His Gly Met
    690                 695                 700

Gln Glu Phe Asp Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe
705                 710                 715                 720

Met Thr Ser Glu His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys
                725                 730                 735

Val Ile Ile Phe Glu Arg Gly Asp Leu Val Phe Val Phe Asn Phe His
            740                 745                 750

Trp Ser Asn Ser Phe Phe Asp Tyr Arg Val Gly Cys Ser Arg Pro Gly
        755                 760                 765

Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Ala Leu Phe Gly Gly Phe
    770                 775                 780

Ser Arg Leu Asp His Asp Val Asp Tyr Phe Thr Thr Glu His Pro His
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785                 790                 795                 800

Asp Asn Arg Pro Arg Ser Phe Ser Val Tyr Thr Pro Ser Arg Thr Ala
                805                 810                 815

Val Val Tyr Ala Leu Thr Glu
            820


<210> SEQ ID NO 2
<211> LENGTH: 823
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 2

Met Ala Thr Phe Ala Val Ser Gly Ala Thr Leu Gly Val Ala Arg Pro
1               5                   10                  15

Ala Ser Ala Gly Gly Gly Leu Leu Arg Ser Gly Ser Glu Arg Arg Gly
            20                  25                  30

Gly Val Asp Leu Pro Ser Leu Leu Leu Arg Lys Lys Asp Ser Ser Arg
        35                  40                  45

Ala Val Leu Ser Arg Ala Ala Ser Pro Gly Lys Val Leu Val Pro Asp
    50                  55                  60

Gly Glu Ser Asp Asp Leu Ala Ala Thr Pro Ala Gln Pro Glu Glu Leu
65                  70                  75                  80

Gln Ile Pro Glu Asp Ile Glu Glu Gln Thr Ala Glu Val Asn Met Thr
                85                  90                  95

Gly Gly Thr Ala Glu Lys Leu Gln Tyr Ser Glu Pro Thr Gln Gly Ile
            100                 105                 110

Val Glu Thr Ile Thr Asp Gly Val Thr Lys Gly Val Lys Glu Leu Val
        115                 120                 125

Val Gly Glu Lys Pro Arg Val Val Pro Lys Pro Gly Asp Gly Gln Lys
    130                 135                 140

Ile Tyr Glu Ile Asp Pro Thr Leu Lys Asp Phe Arg Ser His Leu Asp
145                 150                 155                 160

Tyr Arg Tyr Ser Glu Tyr Lys Arg Ile Arg Ala Ala Ile Asp Gln His
                165                 170                 175

Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys Leu Gly Phe
            180                 185                 190

Thr Arg Ser Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala
        195                 200                 205

His Ser Ala Ala Leu Val Gly Asp Phe Asn Asn Trp Asn Pro Asn Ala
    210                 215                 220

Asp Thr Met Thr Arg Asp Asp Tyr Gly Val Trp Glu Ile Phe Leu Pro
225                 230                 235                 240

Asn Asn Ala Asp Gly Ser Pro Ala Ile Pro His Gly Ser Arg Val Lys
                245                 250                 255

Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile Ser Ala Trp
            260                 265                 270

Ile Lys Phe Ser Val Gln Ala Pro Gly Glu Ile Pro Phe Asn Gly Ile
        275                 280                 285

Tyr Tyr Asp Pro Pro Glu Glu Glu Lys Tyr Val Phe Gln His Pro Gln
    290                 295                 300

Pro Lys Arg Pro Glu Ser Leu Arg Ile Tyr Glu Ser His Ile Gly Met
305                 310                 315                 320

Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Ala Asn Phe Arg Asp Gly
                325                 330                 335
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Val Leu Pro Arg Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met
            340                 345                 350

Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr
        355                 360                 365

Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Glu Asp Leu Lys
    370                 375                 380

Ser Leu Ile Asp Arg Ala His Glu Leu Gly Leu Leu Val Leu Met Asp
385                 390                 395                 400

Ile Val His Ser His Ser Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly
                405                 410                 415

Phe Asp Gly Thr Asp Thr His Tyr Phe His Gly Gly Pro Arg Gly His
            420                 425                 430

His Trp Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Ser Trp Glu Val
        435                 440                 445

Leu Arg Phe Leu Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys
    450                 455                 460

Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met Tyr Thr His
465                 470                 475                 480

His Gly Leu Gln Met Thr Phe Thr Gly Asn Tyr Gly Glu Tyr Phe Gly
                485                 490                 495

Phe Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Val Asn Asp
            500                 505                 510

Leu Ile His Gly Leu Tyr Pro Asp Ala Val Ser Ile Gly Glu Asp Val
        515                 520                 525

Ser Gly Met Pro Thr Phe Cys Ile Pro Val Pro Asp Gly Gly Val Gly
    530                 535                 540

Phe Asp Tyr Arg Leu His Met Ala Val Ala Asp Lys Trp Ile Glu Leu
545                 550                 555                 560

Leu Lys Gln Ser Asp Glu Ser Trp Lys Met Gly Asp Ile Val His Thr
                565                 570                 575

Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser
            580                 585                 590

His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met
        595                 600                 605

Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser Thr Pro
    610                 615                 620

Arg Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Val Thr
625                 630                 635                 640

Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe
                645                 650                 655

Gly His Pro Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Thr Leu Pro
            660                 665                 670

Thr Gly Lys Val Leu Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg
        675                 680                 685

Arg Arg Phe Asp Leu Gly Asp Ala Asp Phe Leu Arg Tyr Arg Gly Met
    690                 695                 700

Gln Glu Phe Asp Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe
705                 710                 715                 720

Met Thr Ser Glu His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys
                725                 730                 735

Val Ile Ile Phe Glu Arg Gly Asp Leu Val Phe Val Phe Asn Phe His
            740                 745                 750

Trp Ser Asn Ser Phe Phe Asp Tyr Arg Val Gly Cys Ser Lys Pro Gly
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        755                 760                 765

Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Ala Leu Phe Gly Gly Phe
    770                 775                 780

Ser Arg Leu Asp His Asp Val Asp Tyr Phe Thr Thr Glu His Pro His
785                 790                 795                 800

Asp Asn Arg Pro Arg Ser Phe Leu Val Tyr Thr Pro Ser Arg Thr Ala
                805                 810                 815

Val Val Tyr Ala Leu Thr Glu
            820


<210> SEQ ID NO 3
<211> LENGTH: 819
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 3

Met Ala Thr Phe Ala Val Ser Gly Ala Thr Leu Gly Val Ala Arg Ala
1               5                   10                  15

Gly Val Gly Val Ala Arg Ala Gly Ser Glu Arg Arg Gly Gly Ala Asp
            20                  25                  30

Leu Pro Ser Leu Leu Leu Arg Lys Lys Asp Ser Ser Arg Ala Val Leu
        35                  40                  45

Ser Arg Ala Ala Ser Pro Gly Lys Val Leu Val Pro Asp Gly Glu Ser
    50                  55                  60

Asp Asp Leu Ala Ser Pro Ala Gln Pro Glu Glu Leu Gln Ile Pro Glu
65                  70                  75                  80

Asp Ile Glu Glu Gln Thr Ala Glu Val Asn Met Thr Gly Gly Thr Ala
                85                  90                  95

Glu Lys Leu Gln Ser Ser Glu Pro Thr Gln Gly Ile Val Glu Thr Ile
            100                 105                 110

Thr Asp Gly Val Thr Lys Gly Val Lys Glu Leu Val Val Gly Glu Lys
        115                 120                 125

Pro Arg Val Val Pro Lys Pro Gly Asp Gly Gln Lys Ile Tyr Glu Ile
    130                 135                 140

Asp Pro Thr Leu Lys Asp Phe Arg Ser His Leu Asp Tyr Arg Tyr Ser
145                 150                 155                 160

Glu Tyr Lys Arg Ile Arg Ala Ala Ile Asp Gln His Glu Gly Gly Leu
                165                 170                 175

Glu Ala Phe Ser Arg Gly Tyr Glu Lys Leu Gly Phe Thr Arg Ser Ala
            180                 185                 190

Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala His Ser Ala Ala
        195                 200                 205

Leu Val Gly Asp Phe Asn Asn Trp Asn Pro Asn Ala Asp Thr Met Thr
    210                 215                 220

Arg Asp Asp Tyr Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp
225                 230                 235                 240

Gly Ser Ser Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp
                245                 250                 255

Thr Pro Ser Gly Val Lys Asp Ser Ile Ser Ala Trp Ile Lys Phe Ser
            260                 265                 270

Val Gln Ala Pro Gly Glu Ile Pro Phe Asn Gly Ile Tyr Tyr Asp Pro
        275                 280                 285

Pro Glu Glu Glu Lys Tyr Val Phe Gln His Pro Gln Arg Lys Arg Pro
    290                 295                 300
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Glu Ser Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser Pro Glu
305                 310                 315                 320

Pro Lys Ile Asn Ser Tyr Ala Asn Phe Arg Asp Glu Val Leu Pro Arg
                325                 330                 335

Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln Glu
            340                 345                 350

His Ser Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala
        355                 360                 365

Pro Ser Ser Arg Phe Gly Thr Pro Glu Asp Leu Lys Ser Leu Ile Asp
    370                 375                 380

Arg Ala His Glu Leu Gly Leu Leu Val Leu Met Asp Ile Val His Ser
385                 390                 395                 400

His Ser Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly Phe Asp Gly Thr
                405                 410                 415

Asp Thr His Tyr Phe His Gly Gly Pro Arg Gly His His Trp Met Trp
            420                 425                 430

Asp Ser Arg Leu Phe Asn Tyr Gly Ser Trp Glu Val Leu Arg Phe Leu
        435                 440                 445

Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys Phe Asp Gly Phe
    450                 455                 460

Arg Phe Asp Gly Val Thr Ser Met Met Tyr Thr His His Gly Leu Gln
465                 470                 475                 480

Met Thr Phe Thr Gly Asn Tyr Gly Glu Tyr Phe Gly Phe Ala Thr Asp
                485                 490                 495

Val Asp Ala Val Val Tyr Leu Met Leu Val Asn Asp Leu Ile His Gly
            500                 505                 510

Leu Tyr Pro Asp Ala Val Ser Ile Gly Glu Asp Val Ser Gly Met Pro
        515                 520                 525

Thr Phe Cys Ile Pro Val Pro Asp Gly Gly Val Gly Phe Asp Tyr Arg
    530                 535                 540

Leu His Met Ala Val Ala Asp Lys Trp Ile Glu Leu Leu Lys Gln Ser
545                 550                 555                 560

Asp Glu Ser Trp Lys Met Gly Asp Ile Val His Thr Leu Thr Asn Arg
                565                 570                 575

Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser His Asp Gln Ala
            580                 585                 590

Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met
        595                 600                 605

Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser Thr Leu Arg Ile Asp Arg
    610                 615                 620

Gly Ile Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly
625                 630                 635                 640

Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu
                645                 650                 655

Trp Ile Asp Phe Pro Arg Gly Pro Gln Thr Leu Pro Thr Gly Lys Val
            660                 665                 670

Leu Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp
        675                 680                 685

Leu Gly Asp Ala Asp Phe Leu Arg Tyr Arg Gly Met Gln Glu Phe Asp
    690                 695                 700

Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe Met Thr Ser Glu
705                 710                 715                 720

His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys Val Ile Ile Leu
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                725                 730                 735

Lys Arg Gly Asp Leu Val Phe Val Phe Asn Phe His Trp Ser Asn Ser
            740                 745                 750

Phe Phe Asp Tyr Arg Val Gly Cys Ser Lys Pro Gly Lys Tyr Lys Val
        755                 760                 765

Ala Leu Asp Ser Asp Asp Ala Leu Phe Gly Gly Phe Ser Arg Leu Asp
    770                 775                 780

His Asp Val Asp Tyr Phe Thr Thr Glu His Pro His Asp Asn Arg Pro
785                 790                 795                 800

Arg Ser Phe Ser Val Tyr Thr Pro Ser Arg Thr Ala Val Val Tyr Ala
                805                 810                 815

Leu Thr Glu


<210> SEQ ID NO 4
<211> LENGTH: 157
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 4

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Ser Ala Pro Arg Ser Gly Gly Pro Glu Arg Arg Gly Arg Gly Val Glu
            20                  25                  30

Leu Gln Ser Pro Ser Leu Leu Phe Gly Arg Asn Lys Gly Thr Arg Ser
        35                  40                  45

Pro Arg Ala Val Gly Val Gly Gly Ser Gly Trp Arg Val Val Met Arg
    50                  55                  60

Ala Gly Gly Pro Ser Gly Glu Val Met Ile Pro Asp Gly Gly Ser Gly
65                  70                  75                  80

Gly Thr Pro Pro Ser Ile His Gly Pro Val Gln Phe Asp Ser Asp Asp
                85                  90                  95

Leu Lys Val Pro Phe Ile Asp Asp Glu Thr Ser Leu Gln Asp Gly Gly
            100                 105                 110

Glu Asp Thr Ile Trp Ser Ser Glu Thr Asn Gln Val Thr Glu Glu Ile
        115                 120                 125

Asp Ala Glu Gly Thr Ser Arg Met Asp Lys Glu Ser Ser Thr Gly Glu
    130                 135                 140

Lys Leu Arg Ile Leu Pro Pro Pro Gly Asn Gly Gln Gln
145                 150                 155


<210> SEQ ID NO 5
<211> LENGTH: 99
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 5

Ala Val Ser Val Gly Gly Ser Gly Trp Arg Val Val Met Arg Ala Gly
1               5                   10                  15

Gly Pro Ser Gly Glu Val Met Ile Pro Asp Gly Gly Ser Gly Gly Thr
            20                  25                  30

Ser Pro Ser Ile Asp Gly Pro Val Gln Phe Asp Ser Asp Asp Leu Lys
        35                  40                  45

Val Pro Phe Ile Asp Asp Glu Pro Ser Leu Gln Asp Glu Gly Glu Asp
    50                  55                  60

Ser Ile Trp Ser Ser Glu Thr Asn Gln Val Thr Glu Glu Ile Asp Val
65                  70                  75                  80
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Glu Gly Met Lys Ile Met Asp Lys Glu Ser Ser Thr Gly Glu Lys Leu
                85                  90                  95

Arg Ile Val


<210> SEQ ID NO 6
<211> LENGTH: 836
<212> TYPE: PRT
<213> ORGANISM: Triticum sp.

<400> SEQUENCE: 6

Met Ala Ala Pro Ala Phe Ala Val Ser Ala Ala Gly Leu Ala Arg Pro
1               5                  10                  15

Ser Ala Pro Arg Ser Gly Gly Ala Glu Arg Arg Gly Arg Gly Val Glu
            20                  25                  30

Leu Gln Ser Pro Ser Leu Leu Phe Gly Arg Asn Lys Gly Thr Arg Ser
        35                  40                  45

Pro Arg Ala Val Gly Val Gly Gly Ser Gly Trp Arg Val Val Met Arg
    50                  55                  60

Ala Gly Gly Pro Ser Gly Glu Val Met Ile Pro Asp Gly Gly Ser Gly
65                  70                  75                  80

Gly Thr Pro Pro Ser Ile Asp Gly Pro Val Gln Phe Asp Ser Asp Asp
                85                  90                  95

Leu Lys Val Pro Phe Ile Asp Asp Glu Thr Ser Leu Gln Asp Gly Gly
            100                 105                 110

Glu Asp Ser Ile Trp Ser Ser Glu Thr Asn Gln Val Ser Glu Glu Ile
        115                 120                 125

Asp Ala Glu Asp Thr Ser Arg Met Asp Lys Glu Ser Ser Thr Arg Glu
    130                 135                 140

Lys Leu Arg Ile Leu Pro Pro Pro Gly Asn Gly Gln Gln Ile Tyr Glu
145                 150                 155                 160

Ile Asp Pro Thr Leu Arg Asp Phe Lys Tyr His Leu Glu Tyr Arg Tyr
                165                 170                 175

Ser Leu Tyr Arg Arg Ile Arg Ser Asp Ile Asp Glu His Glu Gly Gly
            180                 185                 190

Met Asp Val Phe Ser Arg Gly Tyr Glu Lys Phe Gly Phe Met Arg Ser
        195                 200                 205

Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala Asp Ser Ala
    210                 215                 220

Ala Leu Val Gly Asp Phe Asn Asn Trp Asp Pro Asn Ala Asp His Met
225                 230                 235                 240

Ser Lys Asn Asp Leu Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala
                245                 250                 255

Asp Gly Ser Pro Pro Ile Pro His Gly Ser Arg Val Lys Val Arg Met
            260                 265                 270

Gly Thr Pro Ser Gly Thr Lys Asp Ser Ile Pro Ala Trp Ile Lys Tyr
        275                 280                 285

Ser Val Gln Thr Pro Gly Asp Ile Pro Tyr Asn Gly Ile Tyr Tyr Asp
    290                 295                 300

Pro Pro Glu Glu Glu Lys Tyr Val Phe Lys His Pro Gln Pro Lys Arg
305                 310                 315                 320

Pro Lys Ser Leu Arg Ile Tyr Glu Thr His Val Gly Met Ser Ser Pro
                325                 330                 335

Glu Pro Lys Ile Asn Thr Tyr Ala Asn Phe Arg Asp Glu Val Leu Pro
            340                 345                 350
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Arg Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln
        355                 360                 365

Glu His Ser Tyr Tyr Gly Ser Phe Gly Tyr His Val Thr Asn Phe Phe
    370                 375                 380

Ala Pro Ser Ser Arg Phe Gly Ser Pro Glu Asp Leu Lys Ser Leu Ile
385                 390                 395                 400

Asp Arg Ala His Glu Leu Gly Leu Val Val Leu Met Asp Val Val His
                405                 410                 415

Ser His Ala Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly Phe Asp Gly
            420                 425                 430

Thr Asp Thr His Tyr Phe His Gly Gly Ser Arg Gly His His Trp Met
        435                 440                 445

Trp Asp Ser Arg Val Phe Asn Tyr Gly Asn Lys Glu Val Ile Arg Phe
    450                 455                 460

Leu Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys Phe Asp Gly
465                 470                 475                 480

Phe Arg Phe Asp Gly Ala Thr Ser Met Met Tyr Thr His His Gly Leu
                485                 490                 495

Gln Val Thr Phe Thr Gly Ser Tyr His Glu Tyr Phe Gly Phe Ala Thr
            500                 505                 510

Asp Val Asp Ala Val Val Tyr Leu Met Leu Met Asn Asp Leu Ile His
        515                 520                 525

Gly Phe Tyr Pro Glu Ala Val Thr Ile Gly Glu Asp Val Ser Gly Met
    530                 535                 540

Pro Thr Phe Ala Leu Pro Val Gln Val Gly Gly Val Gly Phe Asp Tyr
545                 550                 555                 560

Arg Leu His Met Ala Val Ala Arg Lys Trp Ile Glu Leu Leu Lys Gly
                565                 570                 575

Asn Asp Glu Ala Trp Glu Met Gly Asn Ile Val His Thr Leu Thr Asn
            580                 585                 590

Arg Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser His Asp Gln
        595                 600                 605

Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp
    610                 615                 620

Met Tyr Asp Phe Met Ala Leu Asn Gly Pro Ser Thr Pro Asn Ile Asp
625                 630                 635                 640

Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Ile Thr Met Gly Leu
                645                 650                 655

Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro
            660                 665                 670

Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Val Leu Pro Ser Gly Lys
        675                 680                 685

Phe Ile Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe
    690                 695                 700

Asp Leu Gly Asp Ala Glu Phe Leu Arg Tyr His Gly Met Gln Gln Phe
705                 710                 715                 720

Asp Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe Met Thr Ser
                725                 730                 735

Asp His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys Val Ile Val
            740                 745                 750

Phe Glu Lys Gly Asp Leu Val Phe Val Phe Asn Phe His Trp Ser Ser
        755                 760                 765
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Ser Tyr Phe Asp Tyr Arg Val Gly Cys Leu Lys Pro Gly Lys Tyr Lys
    770                 775                 780

Val Val Leu Asp Ser Asp Ala Gly Leu Phe Gly Gly Phe Gly Arg Ile
785                 790                 795                 800

His His Thr Ala Glu His Phe Thr Ser Asp Cys Gln His Asp Asn Arg
                805                 810                 815

Pro His Ser Phe Ser Val Tyr Thr Pro Ser Arg Thr Cys Val Val Tyr
            820                 825                 830

Ala Pro Met Asn
        835


<210> SEQ ID NO 7
<211> LENGTH: 2966
<212> TYPE: DNA
<213> ORGANISM: Triticum sp.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (35)..(35)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2937)..(2937)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2958)..(2958)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2965)..(2965)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 7

atggtcgacc tgcaggcggc cgcgaatgca ctagngattt tgacaccaga ccaactggta     60

atggtagcga ccggcgctca gctggaattc gcggccgcgt cgaccgtggg tttaagcagg    120

agacgaggcg gggtcagttg ggcagttagg ttggatccga tccggctgcg gcggcggcga    180

cgggatggct gcgccggcat tcgcagtttc cgcggcgggg ctggcccggc cgtcggctcc    240

tcgatccggc ggggcagagc ggaggggggcg cggggtggag ctgcagtcgc catcgctgct    300

cttcggccgc aacaagggca cccgttcacc ccgtgccgtc ggcgtcggag gttctggatg    360

gcgcgtggtc atgcgcgcgg gggggccgtc cggggaggtg atgatccctg acggcggtag    420

tggcggaaca ccgccttcca tcgacggtcc cgttcagttc gattctgatg atctgaaggt    480

tccattcatt gatgatgaaa caagcctaca ggatggaggt gaagatagta tttggtcttc    540

agagacaaat caggttagtg aagaaattga tgctgaagac acgagcagaa tggacaaaga    600

atcatctacg agggagaaat tacgcattct gccaccaccg ggaaatggac agcaaatata    660

cgagattgac ccaacgctcc gagactttaa gtaccatctt gagtatcgat atagcctata    720

caggagaata cgttcagaca ttgatgaaca cgaaggaggc atggatgtat tttcccgcgg    780

ttacgagaag tttggattta tgcgcagcgc tgaaggtatc acttaccgag aatgggctcc    840

tggagcagat tctgcagcat tagttggcga cttcaacaat tgggatccaa atgcagacca    900

tatgagcaaa aatgaccttg gtgtttggga gatttttctg ccaaacaatg cagatggttc    960

gccaccaatt cctcacggct cacgggtgaa ggtgcgaatg ggtactccat ctgggacaaa   1020

ggattcaatt cctgcttgga tcaagtactc cgtgcagact ccaggagata taccatacaa   1080

tggaatatat tatgatcctc ccgaagagga gaagtatgta ttcaagcatc ctcaacctaa   1140

acgaccaaaa tcattgcgga tatatgaaac acatgttggc atgagtagcc cggaaccaaa   1200
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gatcaacaca tatgcaaact tcagggatga ggtgcttcca agaattaaaa gacttggata 1260

caatgcagtg caaataatgg caatccaaga gcactcatac tatggaagct ttgggtacca 1320

tgttaccaat ttctttgcac caagtagccg ttttgggtcc ccagaagatt taaaatcttt 1380

gattgataga gctcacgagc ttggcttggt tgtcctcatg gatgttgttc acagtcacgc 1440

gtcaaataat accttggacg ggttgaatgg ttttgatggc acggatacac attacttcca 1500

tggcggttca cggggccatc actggatgtg ggattcccgt gtgtttaact atgggaataa 1560

ggaagttata aggtttctac tttccaatgc aagatggtgg ctagaggagt ataagtttga 1620

tggtttccga ttcgatggcg cgacctccat gatgtatacc catcatggat tacaagtaac 1680

ctttacagga agctaccatg aatattttgg ctttgccact gatgtagatg cggtcgttta 1740

cttgatgctg atgaatgatc taattcatgg gttttatcct gaagccgtaa ctatcggtga 1800

agatgttagt ggaatgccta catttgccct tcctgttcaa gttggtgggg ttggttttga 1860

ctatcgctta catatggctg ttgcccgcaa atggattgaa cttctcaaag gaaacgatga 1920

agcttgggag atgggtaata ttgtgcacac actaacaaac agaaggtggc tggaaaagtg 1980

tgttacttat gctgaaagtc acgatcaagc acttgttgga gacaagacta ttgcattctg 2040

gttgatggac aaggatatgt atgatttcat ggcgctgaac ggaccttcga cgcctaatat 2100

tgatcgtgga atagcactgc ataaaatgat tagacttatc acaatgggtc taggaggaga 2160

gggttatctt aactttatgg gaaatgagtt cgggcatcct gaatggatag actttccaag 2220

aggcccacaa gtacttccaa gtggtaagtt catcccagga aacaacaaca gttacgacaa 2280

atgccgtcga agatttgacc tgggtgatgc agaatttctt aggtatcatg gtatgcagca 2340

gtttgatcag gcaatgcagc atcttgagga aaaatatggt tttatgacat cagaccacca 2400

gtacgtatct cggaaacatg aggaagataa ggtgatcgtg tttgaaaaag gggacttggt 2460

atttgtgttc aacttccact ggagtagtag ctatttcgac taccgggtcg gctgtttaaa 2520

gcctgggaag tacaaggtgg tcttagactc ggacgctgga ctctttggtg gatttggtag 2580

gatccatcac actgcagagc acttcacttc tgactgccaa catgacaaca ggccccattc 2640

attctcagtg tacactccta gcagaacctg tgttgtctat gctccaatga actaacagca 2700

aagtgcagca tacgcgtgcg cgctgttgtt gctagtagca agaaaaatcg tatggtcaat 2760

acaaccaggt gcaaggttta ataaggattt ttgcttcaac gagtcctgga tagacaagac 2820

aacatgatgt tgtgctgtgt gctcccaatc cccagggcgt tgtgaagaaa acatgctcat 2880

ctgtgttatt ttatggatca gcgacgaaac ctcccccaaa taccccttttt ttttttnaaa 2940

ggaggatagg cccccggnct ttgcnt 2966

<210> SEQ ID NO 8
<211> LENGTH: 695
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 8

Thr Arg Glu Lys Leu Arg Ile Leu Pro Pro Pro Gly Asn Gly Gln Gln
1 5 10 15

Ile Tyr Glu Ile Asp Pro Thr Leu Arg Asp Phe Lys Tyr His Leu Glu
20 25 30

Tyr Arg Tyr Ser Leu Tyr Arg Arg Ile Arg Ser Asp Ile Asp Glu His
35 40 45

Glu Gly Gly Met Asp Val Phe Ser Arg Gly Tyr Glu Lys Phe Gly Phe
50 55 60

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Met Arg Ser Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala
65                  70                  75                  80

Asp Ser Ala Ala Leu Val Gly Asp Phe Asn Asn Trp Asp Pro Asn Ala
                85                  90                  95

Asp His Met Ser Lys Asn Asp Leu Gly Val Trp Glu Ile Phe Leu Pro
            100                 105                 110

Asn Asn Ala Asp Gly Ser Pro Pro Ile Pro His Gly Ser Arg Val Lys
        115                 120                 125

Val Arg Met Asp Thr Pro Ser Gly Ile Lys Asp Ser Ile Pro Ala Trp
    130                 135                 140

Ile Lys Tyr Ser Val Gln Thr Pro Gly Asp Ile Pro Tyr Asn Gly Ile
145                 150                 155                 160

Tyr Tyr Asp Pro Pro Glu Glu Glu Lys Tyr Val Phe Lys His Pro Gln
                165                 170                 175

Pro Lys Arg Pro Lys Ser Leu Arg Ile Tyr Glu Thr His Val Gly Met
            180                 185                 190

Ser Ser Pro Glu Pro Lys Ile Asn Thr Tyr Ala Asn Phe Arg Asp Glu
        195                 200                 205

Val Leu Pro Arg Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met
    210                 215                 220

Ala Ile Gln Glu His Ser Tyr Tyr Gly Ser Phe Gly Tyr His Val Thr
225                 230                 235                 240

Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Ser Pro Glu Asp Leu Lys
                245                 250                 255

Ser Leu Ile Asp Arg Ala His Glu Leu Gly Leu Val Val Leu Met Asp
            260                 265                 270

Val Val His Ser His Ala Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly
        275                 280                 285

Phe Asp Gly Thr Asp Thr His Tyr Phe His Gly Gly Ser Arg Gly His
    290                 295                 300

His Trp Met Trp Asp Ser Arg Val Phe Asn Tyr Gly Asn Lys Glu Val
305                 310                 315                 320

Ile Arg Phe Leu Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys
                325                 330                 335

Phe Asp Gly Phe Arg Phe Asp Gly Ala Thr Ser Met Met Tyr Thr His
            340                 345                 350

His Gly Leu Gln Val Thr Phe Thr Gly Ser Tyr His Glu Tyr Phe Gly
        355                 360                 365

Phe Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Met Asn Asp
    370                 375                 380

Leu Ile His Gly Phe Tyr Pro Glu Ala Val Thr Ile Gly Glu Asp Val
385                 390                 395                 400

Ser Gly Met Pro Thr Phe Ala Leu Pro Val Gln Val Gly Gly Val Gly
                405                 410                 415

Phe Asp Tyr Arg Leu His Met Ala Val Ala Asp Lys Trp Ile Glu Leu
            420                 425                 430

Leu Lys Gly Asn Asp Glu Ala Trp Glu Met Gly Asn Ile Val His Thr
        435                 440                 445

Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser
    450                 455                 460

His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met
465                 470                 475                 480
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Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asn Gly Pro Ser Thr Pro
                485                 490                 495

Asn Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Ile Thr
            500                 505                 510

Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe
        515                 520                 525

Gly His Pro Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Val Leu Pro
    530                 535                 540

Ser Gly Lys Phe Ile Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg
545                 550                 555                 560

Arg Arg Phe Asp Leu Gly Asp Ala Glu Phe Leu Arg Tyr His Gly Met
                565                 570                 575

Gln Gln Phe Asp Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe
            580                 585                 590

Met Thr Ser Asp His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys
        595                 600                 605

Val Ile Val Phe Glu Lys Gly Asp Leu Val Phe Val Phe Asn Phe His
    610                 615                 620

Trp Ser Ser Ser Tyr Phe Asp Tyr Arg Val Gly Cys Leu Lys Pro Gly
625                 630                 635                 640

Lys Tyr Lys Val Val Leu Asp Ser Asp Ala Gly Leu Phe Gly Gly Phe
                645                 650                 655

Gly Arg Ile His His Thr Ala Glu His Val Thr Ser Asp Cys Gln His
            660                 665                 670

Asp Asn Arg Pro His Ser Phe Ser Val Tyr Thr Pro Ser Arg Thr Cys
        675                 680                 685

Val Val Tyr Ala Pro Met Asn
    690                 695


<210> SEQ ID NO 9
<211> LENGTH: 836
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 9

Met Ala Ala Pro Ala Phe Ala Val Ser Ala Ala Gly Leu Ala Arg Pro
1               5                   10                  15

Ser Ala Pro Arg Ser Gly Gly Ala Glu Arg Arg Gly Arg Gly Val Glu
            20                  25                  30

Leu Gln Ser Pro Ser Leu Leu Phe Gly Arg Asn Lys Gly Thr Arg Ser
        35                  40                  45

Pro Arg Ala Val Gly Val Gly Gly Ser Gly Trp Arg Val Val Met Arg
    50                  55                  60

Ala Gly Gly Pro Ser Gly Glu Val Met Ile Pro Asp Gly Gly Ser Gly
65                  70                  75                  80

Gly Thr Pro Pro Ser Ile Asp Gly Pro Val Gln Phe Asp Ser Asp Asp
                85                  90                  95

Leu Lys Val Pro Phe Ile Asp Asp Glu Thr Ser Leu Gln Asp Gly Gly
            100                 105                 110

Glu Asp Ser Ile Trp Ser Ser Glu Thr Asn Gln Val Ser Glu Glu Ile
        115                 120                 125

Asp Ala Glu Asp Thr Ser Arg Met Asp Lys Glu Ser Ser Thr Arg Glu
    130                 135                 140

Lys Leu Arg Ile Leu Pro Pro Pro Gly Asn Gly Gln Gln Ile Tyr Glu
145                 150                 155                 160
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Ile Asp Pro Thr Leu Arg Asp Phe Lys Tyr His Leu Glu Tyr Arg Tyr
                165                 170                 175

Ser Leu Tyr Arg Arg Ile Arg Ser Asp Ile Asp Glu His Glu Gly Gly
            180                 185                 190

Met Asp Val Phe Ser Arg Gly Tyr Glu Lys Phe Gly Phe Met Arg Ser
        195                 200                 205

Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala Asp Ser Ala
    210                 215                 220

Ala Leu Val Gly Asp Phe Asn Asn Trp Asp Pro Asn Ala Asp His Met
225                 230                 235                 240

Ser Lys Asn Asp Leu Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala
                245                 250                 255

Asp Gly Ser Pro Pro Ile Pro His Gly Ser Arg Val Lys Val Arg Met
            260                 265                 270

Asp Thr Pro Ser Gly Ile Lys Asp Ser Ile Pro Ala Trp Ile Lys Tyr
        275                 280                 285

Ser Val Gln Thr Pro Gly Asp Ile Pro Tyr Asn Gly Ile Tyr Tyr Asp
    290                 295                 300

Pro Pro Glu Glu Glu Lys Tyr Val Phe Lys His Pro Gln Pro Lys Arg
305                 310                 315                 320

Pro Lys Ser Leu Arg Ile Tyr Glu Thr His Val Gly Met Ser Ser Pro
                325                 330                 335

Glu Pro Lys Ile Asn Thr Tyr Ala Asn Phe Arg Asp Glu Val Leu Pro
            340                 345                 350

Arg Ile Lys Arg Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln
        355                 360                 365

Glu His Ser Tyr Tyr Gly Ser Phe Gly Tyr His Val Thr Asn Phe Phe
    370                 375                 380

Ala Pro Ser Ser Arg Phe Gly Ser Pro Glu Asp Leu Lys Ser Leu Ile
385                 390                 395                 400

Asp Arg Ala His Glu Leu Gly Leu Val Val Leu Met Asp Val Val His
                405                 410                 415

Ser His Ala Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly Phe Asp Gly
            420                 425                 430

Thr Asp Thr His Tyr Phe His Gly Gly Ser Arg Gly His His Trp Met
        435                 440                 445

Trp Asp Ser Arg Val Phe Asn Tyr Gly Asn Lys Glu Val Ile Arg Phe
    450                 455                 460

Leu Leu Ser Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys Phe Asp Gly
465                 470                 475                 480

Phe Arg Phe Asp Gly Ala Thr Ser Met Met Tyr Thr His His Gly Leu
                485                 490                 495

Gln Val Thr Phe Thr Gly Ser Tyr His Glu Tyr Phe Gly Phe Ala Thr
            500                 505                 510

Asp Val Asp Ala Val Val Tyr Leu Met Leu Met Asn Asp Leu Ile His
        515                 520                 525

Gly Phe Tyr Pro Glu Ala Val Thr Ile Gly Glu Asp Val Ser Gly Met
    530                 535                 540

Pro Thr Phe Ala Leu Pro Val Gln Val Gly Gly Val Gly Phe Asp Tyr
545                 550                 555                 560

Arg Leu His Met Ala Val Ala Asp Lys Trp Ile Glu Leu Leu Lys Gly
                565                 570                 575
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Asn Asp Glu Ala Trp Glu Met Gly Asn Ile Val His Thr Leu Thr Asn
            580                 585                 590

Arg Arg Trp Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser His Asp Gln
        595                 600                 605

Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp
    610                 615                 620

Met Tyr Asp Phe Met Ala Leu Asn Gly Pro Ser Thr Pro Asn Ile Asp
625                 630                 635                 640

Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Ile Thr Met Gly Leu
                645                 650                 655

Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro
            660                 665                 670

Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Val Leu Pro Ser Gly Lys
        675                 680                 685

Phe Ile Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe
    690                 695                 700

Asp Leu Gly Asp Ala Glu Phe Leu Arg Tyr His Gly Met Gln Gln Phe
705                 710                 715                 720

Asp Gln Ala Met Gln His Leu Glu Glu Lys Tyr Gly Phe Met Thr Ser
                725                 730                 735

Asp His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys Val Ile Val
            740                 745                 750

Phe Glu Lys Gly Asp Leu Val Phe Val Phe Asn Phe His Trp Ser Ser
        755                 760                 765

Ser Tyr Phe Asp Tyr Arg Val Gly Cys Leu Lys Pro Gly Lys Tyr Lys
    770                 775                 780

Val Val Leu Asp Ser Asp Ala Gly Leu Phe Gly Gly Phe Gly Arg Ile
785                 790                 795                 800

His His Thr Ala Glu His Val Thr Ser Asp Cys Gln His Asp Asn Arg
                805                 810                 815

Pro His Ser Phe Ser Val Tyr Thr Pro Ser Arg Thr Cys Val Val Tyr
            820                 825                 830

Ala Pro Met Asn
        835


<210> SEQ ID NO 10
<211> LENGTH: 829
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 10

Met Ala Ala Pro Ala Phe Ala Val Ser Ala Ala Gly Ile Ala Arg Pro
1               5                   10                  15

Ser Ala Arg Arg Ser Ser Gly Ala Glu Pro Arg Ser Leu Leu Phe Gly
            20                  25                  30

Arg Asn Lys Gly Thr Arg Phe Pro Arg Ala Val Gly Val Gly Gly Ser
        35                  40                  45

Gly Trp Arg Val Val Met Arg Ala Gly Gly Pro Ser Gly Glu Val Met
    50                  55                  60

Ile Pro Asp Gly Gly Ser Gly Gly Ser Gly Thr Pro Pro Ser Ile Glu
65                  70                  75                  80

Gly Ser Val Gln Phe Glu Ser Asp Asp Leu Glu Val Pro Phe Ile Asp
                85                  90                  95

Asp Glu Pro Ser Leu His Asp Gly Gly Glu Asp Thr Ile Arg Ser Ser
            100                 105                 110
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Glu Thr Tyr Gln Val Thr Glu Glu Ile Asp Ala Glu Gly Val Ser Arg
        115                 120                 125

Met Asp Lys Glu Ser Ser Thr Val Lys Lys Ile Arg Ile Val Pro Gln
    130                 135                 140

Pro Gly Asn Gly Gln Gln Ile Tyr Asp Ile Asp Pro Met Leu Arg Asp
145                 150                 155                 160

Phe Lys Tyr His Leu Glu Tyr Arg Tyr Ser Leu Tyr Arg Arg Ile Arg
                165                 170                 175

Ser Asp Ile Asp Glu Tyr Asp Gly Gly Met Asp Val Phe Ser Arg Gly
            180                 185                 190

Tyr Glu Lys Phe Gly Phe Val Arg Ser Ala Glu Gly Ile Thr Tyr Arg
        195                 200                 205

Glu Trp Ala Pro Gly Ala Asp Ser Ala Ala Leu Val Gly Asp Phe Asn
    210                 215                 220

Asn Trp Asp Pro Thr Ala Asp His Met Ser Lys Asn Asp Leu Gly Ile
225                 230                 235                 240

Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Ser Pro Pro Ile Pro
                245                 250                 255

His Gly Ser Arg Val Lys Val Arg Met Asp Thr Pro Ser Gly Thr Lys
            260                 265                 270

Asp Ser Ile Pro Ala Trp Ile Lys Tyr Ser Val Gln Thr Pro Gly Asp
        275                 280                 285

Ile Pro Tyr Asn Gly Ile Tyr Tyr Asp Pro Pro Glu Glu Glu Lys Tyr
    290                 295                 300

Val Phe Lys His Pro Gln Pro Lys Arg Pro Lys Ser Leu Arg Ile Tyr
305                 310                 315                 320

Glu Thr His Val Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Thr Tyr
                325                 330                 335

Ala Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys Arg Leu Gly Tyr
            340                 345                 350

Asn Ala Val Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Gly Ser
        355                 360                 365

Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly
    370                 375                 380

Ser Pro Glu Asp Leu Lys Ser Leu Ile Asp Arg Ala His Glu Leu Gly
385                 390                 395                 400

Leu Leu Val Leu Met Asp Val Val His Ser His Ala Ser Ser Asn Thr
                405                 410                 415

Leu Asp Gly Leu Asn Gly Phe Asp Gly Thr Asp Thr His Tyr Phe His
            420                 425                 430

Gly Gly Ser Arg Gly His His Trp Met Trp Asp Ser Arg Val Phe Asn
        435                 440                 445

Tyr Gly Asn Lys Glu Val Ile Arg Phe Leu Leu Ser Asn Ala Arg Trp
    450                 455                 460

Trp Leu Glu Glu Tyr Lys Phe Asp Gly Phe Arg Phe Asp Gly Ala Thr
465                 470                 475                 480

Ser Met Met Tyr Thr His His Gly Leu Gln Val Thr Phe Thr Gly Ser
                485                 490                 495

Tyr His Glu Tyr Phe Gly Phe Ala Thr Asp Val Asp Ala Val Val Tyr
            500                 505                 510

Leu Met Leu Val Asn Asp Leu Ile His Ala Leu Tyr Pro Glu Ala Val
        515                 520                 525
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Thr Ile Gly Glu Asp Val Ser Gly Met Pro Thr Phe Ala Leu Pro Val
    530                 535                 540

Gln Val Gly Gly Val Gly Phe Asp Tyr Arg Leu His Met Ala Val Ala
545                 550                 555                 560

Asp Lys Trp Ile Glu Leu Leu Lys Gly Ser Asp Glu Gly Trp Glu Met
                565                 570                 575

Gly Asn Ile Val His Thr Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys
            580                 585                 590

Val Thr Tyr Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr
        595                 600                 605

Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala Leu
    610                 615                 620

Asn Gly Pro Ser Thr Pro Asn Ile Asp Arg Gly Ile Ala Leu His Lys
625                 630                 635                 640

Met Ile Arg Leu Ile Thr Met Ala Leu Gly Gly Glu Gly Tyr Leu Asn
                645                 650                 655

Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg
            660                 665                 670

Gly Pro Gln Val Leu Pro Thr Gly Lys Phe Ile Pro Gly Asn Asn Asn
        675                 680                 685

Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Phe
    690                 695                 700

Leu Arg Tyr His Gly Met Gln Gln Phe Asp Gln Ala Met Gln His Leu
705                 710                 715                 720

Glu Glu Lys Tyr Gly Phe Met Thr Ser Asp His Gln Tyr Val Ser Arg
                725                 730                 735

Lys His Glu Glu Asp Lys Val Ile Val Phe Glu Lys Gly Asp Leu Val
            740                 745                 750

Phe Val Phe Asn Phe His Trp Ser Asn Ser Tyr Phe Asp Tyr Arg Val
        755                 760                 765

Gly Cys Leu Lys Pro Gly Lys Tyr Lys Val Val Leu Asp Ser Asp Ala
    770                 775                 780

Gly Leu Phe Gly Gly Phe Gly Arg Ile His His Thr Gly Glu His Phe
785                 790                 795                 800

Thr Asn Gly Cys Gln His Asp Asn Arg Pro His Ser Phe Ser Val Tyr
                805                 810                 815

Thr Pro Ser Arg Thr Cys Val Val Tyr Ala Pro Met Asn
            820                 825


<210> SEQ ID NO 11
<211> LENGTH: 1267
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 11

catatggctg ttgccgacaa atggattgaa cttctcaaag gaaacgatga agcttgggag     60

atgggtaata ttgtgcacac actaacaaac agaaggtggc tggaaaagtg tgttacttat    120

gctgaaagtc acgatcaagc acttgttgga gacaagacta ttgcattctg gttgatggac    180

aaggatatgt atgatttcat ggcgctgaac ggaccttcga ctgacctaat attgatcgtg    240

gaatagcact gcataaaatg attagactta tcacaatggg tctaggagga gagggttatc    300

ttaactttat gggaaatgag ttcgggcatc ctgaatggat agactttcca agaggcccac    360

aagtacttcc aagtggtaag ttcatcccag gaaacaacaa cagttacgac aaatgccgtc    420
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gaagatttga cctgggtgat gcagaatttc ttaggtatca tggtatgcag cagtttgatc 480

aggcaatgca gcatcttgag gaaaaatatg gttttatgac atcagaccac cagtacgtat 540

ctcggaaaca tgaggaagat aaggtgatcg tgtttgaaaa aggggacttg gtatttgtgt 600

tcaacttcca ctggagtagt agctatttcg actaccgggt cggctgttta aagcctggga 660

agtacaaggt ggtcttagac tcagtacgct ggactctttg gtggatttgg taggatccat 720

cacactgcag agcacttcac ttctgactgc caacatgaca acaggcccca ttcattctca 780

gtgtacactc ctagcagaac ctgtgttgtc tatgctccta tatgaactaa cagcaaagtg 840

cagcatacgc gtgcgcgctg ttgttgctag tagcaagaaa aatcgtatgg tccaatacaa 900

ccaggtgcaa ggtttaataa ggatttttgc ttcaacgagt cctggataga caagacaaca 960

tgatgttgtg ctgtgtgctc cccaatcccc agggcgttgt gaagaaaaca tgctcatctg 1020

tgttatttta tggatcagcg acgaaacctc ccccaaatac ccctttttt tttgaaagga 1080

ggataggccc ccggtctctg catctggatg cctccttaaa tctttgtagc cataaaccat 1140

tgctagtgtc ctctaaattg acagtttaga atagaggttc tacttttgta tcttcttttt 1200

gacagttaga ctgtattcct caaataatcg acatgttgtt tactcgaaga tgagaaataa 1260

aatcaga 1267

<210> SEQ ID NO 12
<211> LENGTH: 1267
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 12

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gctgaaagtc acgatcaagc acttgttgga gacaagacta ttgcattctg gttgatggac 180

aaggatatgt atgatttcat ggcgctgaac ggaccttcga ctgacctaat attgatcgtg 240

gaatagcact gcataaaatg attagactta tcacaatggg tctaggagga gagggttatc 300

ttaactttat gggaaatgag ttcgggcatc ctgaatggat agactttcca agaggcccac 360

aagtacttcc aagtggtaag ttcatcccag gaaacaacaa cagttacgac aaatgccgtc 420

gaagatttga cctgggtgat gcagaatttc ttaggtatca tggtatgcag cagtttgatc 480

aggcaatgca gcatcttgag gaaaaatatg gttttatgac atcagaccac cagtacgtat 540

ctcggaaaca tgaggaagat aaggtgatcg tgtttgaaaa aggggacttg gtatttgtgt 600

tcaacttcca ctggagtagt agctatttcg actaccgggt cggctgttta aagcctggga 660

agtacaaggt ggtcttagac tcagtacgct ggactctttg gtggatttgg taggatccat 720

cacactgcag agcacttcac ttctgactgc caacatgaca acaggcccca ttcattctca 780

gtgtacactc ctagcagaac ctgtgttgtc tatgctccta tatgaactaa cagcaaagtg 840

cagcatacgc gtgcgcgctg ttgttgctag tagcaagaaa aatcgtatgg tccaatacaa 900

ccaggtgcaa ggtttaataa ggatttttgc ttcaacgagt cctggataga caagacaaca 960

tgatgttgtg ctgtgtgctc cccaatcccc agggcgttgt gaagaaaaca tgctcatctg 1020

tgttatttta tggatcagcg acgaaacctc ccccaaatac ccctttttt tttgaaagga 1080

ggataggccc ccggtctctg catctggatg cctccttaaa tctttgtagc cataaaccat 1140

tgctagtgtc ctctaaattg acagtttaga atagaggttc tacttttgta tcttcttttt 1200

gacagttaga ctgtattcct caaataatcg acatgttgtt tactcgaaga tgagaaataa 1260

aatcaga 1267

<210> SEQ ID NO 13
<211> LENGTH: 5832
<212> TYPE: DNA
<213> ORGANISM: T. urartu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3054)..(3054)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3955)..(3955)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 13

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tccattctat ataattgtgc tacatgactt ctttttttctc agatgtatta aaccagttgg 120

acatcaaatg tatttggtac atctagtaaa ctgacagttt caaagaatat cgttttgtaa 180

tggcaacatg atttgatgcc atagatgtgg actgagaagt tcagatgcta tcaagaaaat 240

taatcaactg gccatgtact cgtggcacta catagagttt gcaagttgga aaactgacag 300

caatacctca ctgataagta gctaggcccc acttgccagc ttcatattag atgttacttc 360

cctgttgaac tcatttgaac atattactta aagttcttca tttgtcctaa gtcaaacttc 420

tttaagtttg accaagtcta ctgaaaaata tatcaacatc tacaacacca aattggtttc 480

attagattca caatttttat tttgttatat tagcacacct ttgatgttgt agatatcagc 540

acatttttct acagacttgg tcaaatatag agaagtttga cttaggacaa atctagaact 600

tcaatcaatt tggatcagag gggatagtcc atactggttg attatattcg gtaacatcaa 660

ataatataga tagatgtcaa cactttaaca aaaaatcaga ccttgtcacc aaatatgtat 720

cagaccatct gtttgcttta gccacttgtt ttcatattta tgtgtttgta cctaatctat 780

ttttacttct acttggtttg gttgattttt tttcagttgc attgcttcat caatgatttt 840

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tccacgcggc cacattggat gtgggattct cgtctattca ctatgggagt tgggaagtat 960

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gcttgaagaa tataagtttg atggatttcg atttgatggg gtgacctcca tgatgtatac 1140

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tatttatgat ttttaaccag ttaagtagtt ttatttttggg atcaggctgt tactcttttt 1560

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tcacttaggt aatttgaaaa gtgctaggcc attcaagctt acgagcatat tttttgatgg 1680

ctgtaattta tttgatagta tgcttgtttg ggtttttcag taaatgggag tgtgtgacgg 1740

acatgaaatt atacttcagt gtgttctgta catgtatttg taagagcaag agcaacatgg 1800

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tttaacttaa attcctgcac tgctatggaa tctcactgta tgttgttagt gtacacatcc 1860

gcaaacaagt aatcctgagc tttcaactca tgagaaaata tgaggttcca cttctgccag 1920

cattaactgt tcacagttct aatttgtgta actgtgaaat tgttcaggtc agtggaatgc 1980

ccacattttg catccctgtt ccagatggtg gtgttggttt tgactatcgc ctgcatatgg 2040

ctgtagcaga taaatggatt gaactcctca agtaagtgca ggaatattgg tgattacatg 2100

cgcacaatga tctagattac awtttctaaa tggtaaaaag gaaaatatgt atgtgaatat 2160

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aacttaacta ttcaaccaat ttggtggata ccagagattt ctgccctctt ttcgtaatgt 2760

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tgagaagtgt gtaaacttat gcagaaagtc atgatcaagc actagttggt gacncgacta 3060

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ataaagaata ggataatttg taaagaaaag aatttgttgc tcgagctgtt gtagtcatat 5160

aacatccccg aagcacatct actattcatt catattatct acttaagggt ttgttacaat 5220

ctttgtactc agttggactc actctaatac tggaactatt taccgaatct accctaatca 5280

tcctagcagt tttagagcag ccccatttgg acagtccact gggtttagtt ggtttgtgac 5340

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ttgactcgtc ttgactcaac tgcttacaaa tctgaatcaa cttctcattt gctgatgccc 5520

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tctaagcgcc atgactggga ggggatcgtg cctcttcccc agatgccagg aggagcagat 5760

ggataggtag cttgttggtg agcgctcgaa agaaaatgga cgggcctggg tgtttgtcgt 5820

gctgcactac cc 5832

<210> SEQ ID NO 14
<211> LENGTH: 739
<212> TYPE: PRT
<213> ORGANISM: T. urartu

<400> SEQUENCE: 14

Cys Thr Cys Thr Gly Cys Gly Cys Gly Cys Gly Cys Ala Thr Gly Gly
1 5 10 15

Cys Cys Thr Gly Thr Thr Cys Gly Ala Thr Gly Cys Thr Gly Thr Thr
20 25 30

Cys Cys Cys Cys Ala Gly Thr Thr Gly Ala Thr Cys Thr Cys Cys Ala
35 40 45

Thr Cys Ala Ala Cys Gly Ala Gly Ala Gly Ala Gly Ala Thr Ala Gly
50 55 60

Cys Thr Gly Gly Ala Thr Thr Ala Gly Gly Cys Gly Ala Thr Cys Gly
65 70 75 80

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Cys Cys Thr Gly Cys Gly Thr Cys Ala Gly Thr Gly Thr Cys Ala Cys
                85                  90                  95

Cys Cys Ala Gly Gly Cys Cys Cys Thr Gly Gly Thr Gly Thr Thr Ala
            100                 105                 110

Thr Cys Ala Cys Gly Gly Cys Thr Thr Thr Gly Ala Thr Cys Ala Thr
        115                 120                 125

Cys Thr Cys Cys Thr Cys Cys Thr Cys Cys Cys Ala Thr Thr Cys Thr
    130                 135                 140

Gly Ala Thr Ala Thr Thr Thr Thr Cys Thr Cys Ala Cys Thr Cys Thr
145                 150                 155                 160

Thr Thr Cys Thr Thr Cys Thr Gly Thr Thr Cys Thr Thr Gly Cys Thr
                165                 170                 175

Gly Thr Ala Ala Cys Thr Gly Cys Ala Ala Gly Thr Thr Gly Thr Ala
            180                 185                 190

Gly Cys Ala Thr Thr Gly Thr Cys Thr Cys Ala Cys Thr Ala Thr Thr
        195                 200                 205

Gly Thr Ala Gly Thr Cys Ala Thr Cys Cys Thr Thr Gly Cys Ala Thr
    210                 215                 220

Thr Thr Gly Cys Ala Gly Gly Cys Gly Cys Cys Gly Thr Cys Cys Thr
225                 230                 235                 240

Gly Ala Gly Cys Cys Gly Cys Gly Cys Gly Gly Cys Cys Thr Cys Thr
                245                 250                 255

Cys Cys Ala Gly Gly Gly Ala Ala Gly Gly Thr Cys Cys Thr Gly Gly
            260                 265                 270

Thr Gly Cys Cys Thr Gly Ala Cys Gly Gly Thr Gly Ala Gly Ala Gly
        275                 280                 285

Cys Gly Ala Cys Gly Ala Cys Thr Thr Gly Gly Cys Ala Ala Gly Thr
    290                 295                 300

Cys Cys Gly Gly Cys Gly Cys Ala Ala Cys Cys Thr Gly Ala Ala Gly
305                 310                 315                 320

Ala Ala Thr Thr Ala Cys Ala Gly Gly Thr Ala Cys Ala Cys Ala Cys
                325                 330                 335

Cys Ala Thr Cys Gly Thr Gly Cys Cys Gly Gly Gly Ala Ala Ala Thr
            340                 345                 350

Cys Thr Thr Cys Ala Thr Ala Cys Ala Ala Thr Cys Gly Thr Thr Ala
        355                 360                 365

Thr Thr Cys Ala Cys Thr Thr Ala Cys Cys Ala Ala Ala Thr Gly Cys
    370                 375                 380

Cys Gly Gly Ala Thr Gly Ala Ala Ala Cys Cys Ala Ala Gly Cys Cys
385                 390                 395                 400

Gly Cys Gly Gly Ala Gly Gly Cys Gly Thr Cys Ala Gly Gly Thr Thr
                405                 410                 415

Thr Thr Gly Ala Gly Cys Thr Thr Cys Thr Thr Cys Thr Ala Thr Cys
            420                 425                 430

Ala Gly Cys Ala Thr Thr Gly Thr Gly Cys Ala Gly Thr Ala Cys Thr
        435                 440                 445

Gly Cys Ala Cys Thr Gly Cys Cys Thr Thr Gly Thr Gly Cys Ala Thr
    450                 455                 460

Thr Thr Thr Gly Thr Thr Ala Gly Cys Cys Gly Thr Gly Gly Cys Cys
465                 470                 475                 480

Cys Cys Gly Thr Gly Cys Thr Gly Gly Cys Thr Cys Thr Thr Gly Gly
                485                 490                 495
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Gly Cys Cys Ala Cys Thr Gly Ala Ala Ala Ala Cys Thr Cys Ala Gly
            500                 505                 510

Ala Thr Gly Gly Ala Thr Gly Thr Gly Cys Ala Thr Thr Cys Thr Ala
        515                 520                 525

Gly Cys Ala Ala Gly Ala Ala Cys Thr Thr Cys Ala Cys Gly Ala Ala
    530                 535                 540

Ala Thr Ala Ala Thr Gly Cys Ala Cys Thr Gly Thr Thr Thr Gly Thr
545                 550                 555                 560

Gly Gly Thr Thr Thr Cys Gly Thr Thr Ala Gly Thr Cys Thr Gly Cys
                565                 570                 575

Thr Cys Thr Ala Cys Ala Ala Thr Thr Gly Cys Thr Ala Thr Thr Thr
            580                 585                 590

Thr Thr Cys Gly Thr Gly Cys Thr Gly Thr Ala Gly Ala Thr Ala Cys
        595                 600                 605

Cys Thr Gly Ala Ala Gly Ala Cys Ala Thr Cys Gly Ala Gly Gly Ala
    610                 615                 620

Gly Cys Ala Ala Ala Cys Gly Gly Cys Thr Gly Ala Ala Gly Thr Ala
625                 630                 635                 640

Ala Ala Cys Ala Thr Gly Ala Cys Ala Gly Gly Gly Gly Gly Gly Ala
                645                 650                 655

Cys Thr Gly Cys Ala Gly Ala Ala Ala Ala Ala Cys Thr Thr Gly Ala
            660                 665                 670

Ala Thr Cys Thr Thr Cys Ala Gly Ala Ala Cys Cys Gly Ala Cys Thr
        675                 680                 685

Cys Ala Ala Gly Gly Cys Ala Thr Thr Gly Thr Gly Gly Ala Ala Ala
    690                 695                 700

Cys Ala Ala Thr Cys Ala Cys Thr Gly Ala Thr Gly Gly Thr Gly Thr
705                 710                 715                 720

Ala Ala Cys Cys Ala Ala Ala Gly Gly Ala Gly Thr Thr Ala Ala Gly
                725                 730                 735

Gly Ala Ala


<210> SEQ ID NO 15
<211> LENGTH: 1517
<212> TYPE: PRT
<213> ORGANISM: T. urartu

<400> SEQUENCE: 15

Thr Thr Gly Ala Cys Thr Ala Cys Cys Gly Gly Thr Ala Ala Thr Gly
1               5                   10                  15

Cys Cys Thr Ala Cys Cys Cys Gly Cys Thr Ala Cys Thr Thr Thr Cys
            20                  25                  30

Gly Cys Thr Cys Ala Thr Thr Thr Thr Gly Ala Ala Thr Thr Ala Ala
        35                  40                  45

Gly Gly Thr Cys Cys Thr Thr Thr Cys Gly Thr Cys Ala Thr Gly Cys
    50                  55                  60

Ala Ala Ala Thr Thr Thr Gly Gly Gly Gly Ala Ala Cys Ala Thr Cys
65                  70                  75                  80

Ala Ala Ala Gly Ala Gly Ala Cys Ala Ala Ala Gly Ala Cys Thr Ala
                85                  90                  95

Gly Gly Gly Ala Cys Cys Ala Cys Thr Ala Thr Thr Thr Cys Thr Thr
            100                 105                 110

Ala Cys Ala Gly Thr Thr Cys Cys Cys Cys Thr Cys Ala Thr Gly Gly
        115                 120                 125
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Thr Cys Thr Gly Ala Gly Ala Ala Thr Ala Thr Gly Cys Thr Gly Gly
    130                 135                 140

Gly Ala Cys Gly Thr Ala Gly Ala Thr Gly Thr Ala Thr Ala Ala Thr
145                 150                 155                 160

Thr Gly Ala Thr Gly Gly Cys Thr Ala Cys Ala Ala Thr Thr Thr Gly
                165                 170                 175

Cys Thr Cys Ala Thr Ala Ala Thr Thr Ala Cys Gly Ala Thr Ala Cys
            180                 185                 190

Ala Ala Ala Thr Ala Ala Cys Thr Gly Thr Cys Thr Cys Thr Gly Ala
        195                 200                 205

Thr Cys Ala Thr Thr Gly Cys Ala Ala Thr Thr Ala Cys Ala Gly Ala
    210                 215                 220

Gly Thr Gly Gly Cys Ala Ala Ala Cys Thr Gly Ala Thr Thr Ala Ala
225                 230                 235                 240

Ala Ala Thr Gly Thr Gly Ala Thr Ala Gly Ala Thr Gly Gly Gly Thr
                245                 250                 255

Thr Ala Thr Ala Gly Ala Thr Thr Thr Thr Ala Cys Thr Thr Thr Gly
            260                 265                 270

Cys Thr Ala Ala Thr Thr Cys Cys Thr Cys Thr Ala Cys Cys Ala Ala
        275                 280                 285

Ala Thr Thr Cys Cys Thr Gly Gly Gly Gly Ala Ala Ala Ala Ala Ala
    290                 295                 300

Ala Thr Cys Thr Ala Cys Cys Ala Gly Thr Thr Gly Gly Gly Cys Ala
305                 310                 315                 320

Ala Cys Thr Thr Ala Gly Thr Thr Thr Cys Thr Thr Ala Thr Cys Thr
                325                 330                 335

Thr Thr Gly Thr Thr Gly Cys Cys Thr Cys Thr Thr Thr Gly Thr Thr
            340                 345                 350

Thr Thr Gly Gly Gly Gly Ala Ala Ala Ala Cys Ala Cys Ala Cys Thr
        355                 360                 365

Gly Cys Thr Ala Ala Ala Thr Thr Thr Gly Ala Ala Thr Gly Ala Thr
    370                 375                 380

Thr Thr Thr Gly Gly Gly Thr Ala Thr Gly Cys Cys Thr Cys Cys Gly
385                 390                 395                 400

Thr Gly Gly Ala Thr Thr Cys Ala Ala Cys Ala Gly Ala Thr Ala Cys
                405                 410                 415

Ala Gly Cys Gly Ala Ala Thr Ala Cys Ala Gly Gly Ala Gly Ala Ala
            420                 425                 430

Thr Thr Cys Gly Thr Gly Cys Thr Gly Cys Thr Ala Thr Thr Gly Ala
        435                 440                 445

Cys Cys Ala Ala Cys Ala Thr Gly Ala Ala Gly Gly Thr Gly Gly Ala
    450                 455                 460

Thr Thr Gly Gly Ala Ala Gly Cys Ala Thr Thr Thr Thr Cys Thr Cys
465                 470                 475                 480

Gly Thr Gly Gly Thr Thr Ala Thr Gly Ala Ala Ala Ala Gly Cys Thr
                485                 490                 495

Thr Gly Gly Ala Thr Thr Thr Ala Cys Cys Cys Gly Cys Ala Gly Gly
            500                 505                 510

Thr Ala Ala Ala Thr Thr Thr Ala Ala Ala Gly Cys Thr Thr Cys Ala
        515                 520                 525

Gly Thr Ala Thr Thr Ala Thr Gly Ala Ala Gly Cys Gly Cys Cys Thr
    530                 535                 540

Cys Cys Ala Cys Thr Ala Gly Thr Cys Thr Ala Cys Thr Thr Gly Cys
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```
545                 550                 555                 560

Ala Thr Ala Thr Cys Thr Thr Ala Cys Ala Ala Gly Ala Ala Ala Ala
                565                 570                 575

Thr Thr Thr Ala Thr Ala Ala Thr Thr Cys Cys Thr Gly Thr Thr Thr
            580                 585                 590

Thr Cys Gly Cys Cys Thr Cys Thr Cys Thr Thr Thr Thr Thr Thr Cys
        595                 600                 605

Cys Ala Gly Thr Gly Cys Thr Gly Ala Ala Gly Gly Thr Ala Thr Thr
    610                 615                 620

Gly Thr Cys Thr Ala Gly Thr Thr Gly Cys Ala Thr Ala Thr Cys Thr
625                 630                 635                 640

Thr Ala Thr Ala Ala Gly Ala Ala Ala Ala Thr Thr Thr Ala Thr Gly
                645                 650                 655

Thr Thr Cys Cys Thr Gly Thr Thr Thr Thr Cys Cys Cys Cys Thr Ala
            660                 665                 670

Thr Thr Thr Thr Cys Cys Ala Gly Thr Gly Cys Thr Gly Ala Ala Gly
        675                 680                 685

Gly Thr Ala Thr Cys Ala Cys Thr Thr Ala Cys Cys Gly Ala Gly Ala
    690                 695                 700

Ala Thr Gly Gly Gly Cys Thr Cys Cys Thr Gly Gly Ala Gly Cys Gly
705                 710                 715                 720

Cys Ala Thr Gly Thr Ala Cys Gly Thr Cys Thr Thr Thr Thr Ala Ala
                725                 730                 735

Gly Thr Cys Thr Thr Ala Ala Cys Ala Gly Ala Cys Ala Cys Cys Thr
            740                 745                 750

Thr Cys Cys Ala Ala Thr Thr Cys Ala Thr Thr Gly Thr Thr Ala Ala
        755                 760                 765

Thr Gly Gly Thr Cys Ala Cys Ala Cys Thr Thr Ala Thr Thr Cys Ala
    770                 775                 780

Cys Cys Ala Ala Cys Thr Ala Gly Cys Thr Thr Ala Cys Thr Gly Gly
785                 790                 795                 800

Ala Cys Thr Thr Ala Cys Ala Ala Cys Thr Thr Ala Gly Cys Thr Thr
                805                 810                 815

Ala Cys Thr Gly Ala Ala Thr Ala Cys Thr Gly Ala Cys Cys Ala Gly
            820                 825                 830

Thr Thr Gly Cys Thr Cys Thr Ala Ala Ala Thr Thr Thr Ala Thr Gly
        835                 840                 845

Ala Thr Cys Thr Gly Gly Cys Thr Thr Thr Thr Gly Cys Ala Thr Cys
    850                 855                 860

Cys Thr Ala Thr Thr Ala Cys Ala Gly Thr Cys Thr Gly Cys Ala Gly
865                 870                 875                 880

Cys Ala Thr Thr Ala Gly Thr Ala Gly Gly Thr Gly Ala Cys Thr Thr
                885                 890                 895

Cys Ala Ala Cys Ala Ala Thr Thr Gly Gly Ala Ala Thr Cys Cys Gly
            900                 905                 910

Ala Ala Thr Gly Cys Ala Gly Ala Thr Ala Cys Thr Ala Thr Gly Ala
        915                 920                 925

Cys Cys Ala Gly Ala Gly Thr Ala Thr Gly Thr Cys Thr Ala Cys Ala
    930                 935                 940

Gly Cys Thr Thr Gly Gly Cys Ala Ala Thr Cys Thr Thr Cys Cys Ala
945                 950                 955                 960

Cys Cys Thr Thr Thr Gly Cys Thr Thr Cys Ala Thr Ala Ala Cys Thr
                965                 970                 975
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Ala Cys Thr Gly Ala Thr Ala Cys Ala Thr Cys Thr Ala Thr Thr Thr
            980                 985                 990

Gly Thr Ala Thr Thr Thr Ala Thr  Thr Thr Thr Gly Cys  Thr Gly Thr
        995                1000                1005

Thr Thr  Gly Cys Ala Cys Ala  Thr Thr Cys Cys Thr  Thr Ala Ala
    1010                1015                1020

Ala Gly  Thr Thr Gly Ala Gly  Cys Cys Thr Cys Ala  Ala Cys Thr
    1025                1030                1035

Ala Thr  Ala Thr Cys Ala Thr  Ala Thr Cys Ala Ala  Ala Ala Thr
    1040                1045                1050

Gly Gly  Thr Ala Thr Ala Ala  Thr Thr Thr Gly Thr  Cys Ala Gly
    1055                1060                1065

Thr Gly  Thr Cys Thr Thr Ala  Ala Gly Cys Thr Thr  Cys Ala Gly
    1070                1075                1080

Cys Cys  Thr Ala Ala Ala Gly  Ala Thr Thr Cys Thr  Ala Cys Thr
    1085                1090                1095

Gly Ala  Ala Ala Thr Thr Gly  Gly Thr Cys Cys Ala  Thr Cys Thr
    1100                1105                1110

Thr Thr  Thr Thr Gly Ala Gly  Ala Thr Thr Gly Ala  Ala Ala Ala
    1115                1120                1125

Thr Gly  Ala Gly Thr Ala Thr  Ala Thr Thr Ala Ala  Gly Gly Ala
    1130                1135                1140

Thr Gly  Ala Ala Thr Gly Ala  Ala Thr Ala Gly Gly  Thr Gly Cys
    1145                1150                1155

Ala Ala  Cys Ala Cys Thr Cys  Cys Cys Ala Thr Thr  Cys Thr Thr
    1160                1165                1170

Thr Gly  Gly Thr Ala Gly Ala  Ala Cys Cys Thr Thr  Cys Thr Ala
    1175                1180                1185

Cys Ala  Thr Thr Ala Thr Gly  Thr Gly Thr Gly Thr  Thr Thr Thr
    1190                1195                1200

Thr Tyr  Cys Ala Thr Cys Thr  Ala Cys Ala Ala Thr  Gly Ala Gly
    1205                1210                1215

Cys Ala  Thr Ala Thr Thr Thr  Cys Cys Ala Thr Gly  Cys Thr Ala
    1220                1225                1230

Thr Cys  Ala Gly Thr Gly Ala  Ala Gly Gly Thr Thr  Thr Gly Cys
    1235                1240                1245

Thr Cys  Cys Thr Ala Thr Thr  Gly Ala Thr Gly Cys  Cys Gly Ala
    1250                1255                1260

Thr Ala  Thr Thr Thr Gly Ala  Thr Ala Thr Gly Ala  Thr Cys Thr
    1265                1270                1275

Thr Thr  Thr Cys Ala Gly Gly  Ala Thr Gly Ala Thr  Thr Ala Thr
    1280                1285                1290

Gly Gly  Thr Gly Thr Thr Thr  Gly Gly Gly Ala Gly  Ala Thr Thr
    1295                1300                1305

Thr Thr  Cys Cys Thr Cys Cys  Cys Thr Ala Ala Cys  Ala Ala Thr
    1310                1315                1320

Gly Cys  Thr Gly Ala Thr Gly  Gly Ala Thr Cys Cys  Cys Cys Ala
    1325                1330                1335

Gly Cys  Thr Ala Thr Thr Cys  Cys Thr Cys Ala Thr  Gly Gly Cys
    1340                1345                1350

Thr Cys  Ala Cys Gly Thr Gly  Thr Ala Ala Ala Gly  Gly Thr Ala
    1355                1360                1365
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Ala Gly  Cys Thr Gly Gly Cys  Cys Ala Ala Thr Thr  Ala Thr Thr
    1370                1375                1380

Thr Ala  Gly Thr Thr Gly Ala  Gly Gly Ala Thr Gly  Thr Ala Gly
    1385                1390                1395

Cys Ala  Thr Thr Thr Thr Cys  Gly Ala Ala Cys Thr  Cys Thr Gly
    1400                1405                1410

Cys Cys  Cys Ala Cys Thr Ala  Ala Gly Gly Gly Thr  Cys Cys Cys
    1415                1420                1425

Thr Thr  Thr Gly Cys Cys Thr  Thr Thr Cys Thr Gly  Thr Thr Thr
    1430                1435                1440

Thr Cys  Thr Ala Gly Ala Thr  Ala Cys Gly Gly Ala  Thr Gly Gly
    1445                1450                1455

Ala Thr  Ala Cys Thr Cys Cys  Ala Thr Cys Thr Gly  Gly Thr Gly
    1460                1465                1470

Thr Gly  Ala Ala Gly Gly Ala  Thr Thr Cys Ala Ala  Thr Thr Thr
    1475                1480                1485

Cys Thr  Gly Cys Thr Thr Gly  Gly Ala Thr Cys Ala  Ala Gly Thr
    1490                1495                1500

Thr Cys  Thr Cys Thr Gly Thr  Gly Cys Ala Gly Gly  Cys Thr
    1505                1510                1515


<210> SEQ ID NO 16
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 16

Ala Ala Ser Pro Gly Lys Val Leu Val Pro Asp Gly Glu Ser Asp Asp
1               5                   10                  15

Leu


<210> SEQ ID NO 17
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 17

Ala Gly Gly Pro Ser Gly Glu Val Met Ile
1               5                   10


<210> SEQ ID NO 18
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 18

Val Ser Ala Pro Arg Asp Tyr Thr Met Ala Thr Ala Glu Asp Gly Val
1               5                   10                  15


<210> SEQ ID NO 19
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 19

cccgctgctt tcgctcattt tg                                        22


<210> SEQ ID NO 20
<211> LENGTH: 22
<212> TYPE: DNA
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<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 20

gactaccgga gctcccacct tc 22

<210> SEQ ID NO 21
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 21

cattcgtcaa ataataccct tgacgg 26

<210> SEQ ID NO 22
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 22

cttcaccaat ggatacagca tcag 24

<210> SEQ ID NO 23
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 23

ggtaccggca aatatacgag attgacccg 29

<210> SEQ ID NO 24
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 24

gagctcccac cttcatgttg gtcaatagc 29

<210> SEQ ID NO 25
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 25

gatacctgaa gatatcgagg agc 23

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 26

cggtagtcaa gatggctccg 20

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 27

cacacgttgc tccccttct c 21

<210> SEQ ID NO 28
<211> LENGTH: 23

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<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 28

gagaggagtc cttcttcctg agg 23

<210> SEQ ID NO 29
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 29

gtacaatttt acctgatgag atcatgg 27

<210> SEQ ID NO 30
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 30

cttcaggaat ggatacagca tcag 24

<210> SEQ ID NO 31
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 31

acgatgcact ctttggtgga t 21

<210> SEQ ID NO 32
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 32

acttacggtt gtgaagtagt cgacat 26

<210> SEQ ID NO 33
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 33

cagcaggctt gatcat 16

<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 34

gatccgaata gctggctcaa gtat 24

<210> SEQ ID NO 35
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 35

ggagactgca ggtagggatc aac 23

<210> SEQ ID NO 36

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<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 36

gttcgatgct gttccccag 19

<210> SEQ ID NO 37
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 37

agccgtttgc tcctcgatg 19

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 38

ttccccagtt gatctccatc 20

<210> SEQ ID NO 39
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 39

cttactgaat actgaccagt tg 22

<210> SEQ ID NO 40
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 40

tttatgatct ggcttttgca tccta 25

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 41

gatgttcccc aaatttgcat gac 23

<210> SEQ ID NO 42
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 42

aatgcacaag gcagtgaagt ag 22

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 43

cccaattgat ctccatgagt 20

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<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 44

aaccccaaac ggtgcattat g 21

<210> SEQ ID NO 45
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 45

cggctttgat cattcctcg 19

<210> SEQ ID NO 46
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 46

gctagaatgc acatccatct gat 23

<210> SEQ ID NO 47
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 47

gtaactgcaa gttgtggcg 19

<210> SEQ ID NO 48
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 48

gcttactgaa tactgaccag ttacta 26

<210> SEQ ID NO 49
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 49

ccttaattca aaatgagcga aagc 24

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 50

ggctaactgt tcctgttaaa 20

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 51

gatgagatca tggacgattc 20

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<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 52

Ala Ala Thr Ala Ala Ala Thr Ala Ala Thr Ala Ala Thr Cys Ala Cys
1 5 10 15

Thr Thr Cys Gly
20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 53

gagtaacagc ctgatcccaa 20

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 54

taacaaaaag agtaacagcc 20

<210> SEQ ID NO 55
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 55

gtcaatctgt tcttacacg 19

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 56

caaaaagagt agtaacagct 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 57

caaggtataa attagcattc 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 58

gttttatttt ggggatcagt 20

<210> SEQ ID NO 59
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

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<400> SEQUENCE: 59

ccctaacaaa aagtgtaaca ga 22

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 60

atcagacctt gtcaccaaat 20

<210> SEQ ID NO 61
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 61

gcacttacat cttcaccaat g 21

<210> SEQ ID NO 62
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 62

gccttctgaa gcaattgaca ag 22

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 63

ggctaactgt tcctgttaaa 20

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 64

cgacatgtgt aagaacagat 20

<210> SEQ ID NO 65
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 65

gtcgatattc tattcttatg t 21

<210> SEQ ID NO 66
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 66

ctttttttagg gcactgaaat 20

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 67

gttatgatgc atagcaatta 20

<210> SEQ ID NO 68
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 68

tcttagatag ttccctagta c 21

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 69

caggtaaaat tgtacaagcg 20

<210> SEQ ID NO 70
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 70

acctgatgag atcatggac 19

<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 71

tacctgatga gatcatggac 20

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 72

gatgagatca tggacgattc 20

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 73

aataaataat aatcacttcg 20

<210> SEQ ID NO 74
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 74

tctttttgtt aggggtaag 19

<210> SEQ ID NO 75
<211> LENGTH: 19

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<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 75

agtttgacca agtctactg 19

<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 76

atcagacctt gtcaccaaat 20

<210> SEQ ID NO 77
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 77

gcacttacat cttcaccaat g 21

<210> SEQ ID NO 78
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 78

gtagttataa gcaatatg 18

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 79

catcaagtgg tttcagtaac 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 80

gttactgaaa ccacttgatg 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 81

ttgggatcag gctgttactc 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 82

gagtaacagc ctgatcccaa 20

<210> SEQ ID NO 83

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 83

ggctgttact ctttttgtta 20

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 84

taacaaaaag agtaacagcc 20

<210> SEQ ID NO 85
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 85

ttaaccagtt aagtagtt 18

<210> SEQ ID NO 86
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 86

aactacttaa ctggttaa 18

<210> SEQ ID NO 87
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 87

gatcccaaaa taaaactact t 21

<210> SEQ ID NO 88
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 88

cccaaaataa aactactt 18

<210> SEQ ID NO 89
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 89

gtgggattct cgtctg 16

<210> SEQ ID NO 90
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 90

ttgggaagta tgtagctgc 19

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<210> SEQ ID NO 91
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 91

ttggctaact gttcctgtc 19

<210> SEQ ID NO 92
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 92

gtcaatctgt tcttacacg 19

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 93

atctgttctt acacgtgtca 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 94

gtcaatattc tattcttata 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 95

ctattcttat acaggtatta 20

<210> SEQ ID NO 96
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 96

aacgcgagat ggtggcttga t 21

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 97

caagtggttt cagtaacttc 20

<210> SEQ ID NO 98
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 98

tggtttcagt aacttcttc 19

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<210> SEQ ID NO 99
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 99

ggaagattgg aagtgattg 19

<210> SEQ ID NO 100
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 100

tggaagtgat tgttattat 19

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 101

ttgcttcttg ttctagatgg 20

<210> SEQ ID NO 102
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 102

ttcccaactc ccatagtgaa c 21

<210> SEQ ID NO 103
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 103

caaatatggt gacagaagtc g 21

<210> SEQ ID NO 104
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 104

cacgtgtaag aacagattg 19

<210> SEQ ID NO 105
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 105

agaatagaat attgacac 18

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 106

gtaagaatct taatacctgt 20

<210> SEQ ID NO 107
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 107

cgcgtttgac agtaagaatc tt 22

<210> SEQ ID NO 108
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 108

ccatcaaact tatattca 18

<210> SEQ ID NO 109
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 109

caattgtttc agtgccctga ag 22

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 110

gcaattgttt cagtgccctg 20

<210> SEQ ID NO 111
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 111

cttagaagaa aaaataataa c 21

<210> SEQ ID NO 112
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 112

gcaaacttag aagaaaaaa 19

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 113

ccatagttcc cagtaaatgc 20

<210> SEQ ID NO 114
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 114

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ctactattaa attaactg 18

<210> SEQ ID NO 115
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 115

atccccaaaa taaaactact at 22

<210> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 116

caaaaagagt agtaacagct 20

<210> SEQ ID NO 117
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 117

caaggtataa attagcattc 20

<210> SEQ ID NO 118
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 118

gcattcttat gaaaagac 18

<210> SEQ ID NO 119
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 119

tctgttctta cacatgtt 18

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 120

ctttttttagg gcactgaaac 20

<210> SEQ ID NO 121
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 121

gattattatt tattttcctt ctaagtttgt 30

<210> SEQ ID NO 122
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 122

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acctgatgag atcatggaag attg 24

<210> SEQ ID NO 123
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 123

gtgattatta tttattttc 19

<210> SEQ ID NO 124
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 124

ttattttcct tctaagtttg t 21

<210> SEQ ID NO 125
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 125

gtgattatta tttattttc 19

<210> SEQ ID NO 126
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 126

tgatgcggta gtttacttga tgt 23

<210> SEQ ID NO 127
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 127

gatttttaac tagttaagta gtt 23

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 128

gttttatttt ggggatcagt 20

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 129

cctgcataag aatagaatat ca 22

<210> SEQ ID NO 130
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 130

catgttatga tgcatagcaa ttg 23

<210> SEQ ID NO 131
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 131

gtaaatgtca tctagaacaa gaaa 24

<210> SEQ ID NO 132
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 132

caagaaacaa acttagaagg 20

<210> SEQ ID NO 133
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 133

acaaacttag aaggaaaata a 21

<210> SEQ ID NO 134
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 134

catcagtagc aaatccaaaa tat 23

<210> SEQ ID NO 135
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 135

accccgtaat tattggcgct 20

<210> SEQ ID NO 136
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 136

actctgatga tctgaaggta g 21

<210> SEQ ID NO 137
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 137

tcatgcaggc aggtactag 19

<210> SEQ ID NO 138
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

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<400> SEQUENCE: 138

gtggcagaat gcgtaatttc tct 23

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 139

cagcgatctt acgttcccta 20

<210> SEQ ID NO 140
<211> LENGTH: 19
<212> TYPE: DNA
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<400> SEQUENCE: 140

atgtctgtag gtgccgtca 19

<210> SEQ ID NO 141
<211> LENGTH: 25
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<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 141

caacaaatta gaaagaggat attcc 25

<210> SEQ ID NO 142
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 142

ccgtagatga ttctttgtcc atta 24

<210> SEQ ID NO 143
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 143

atggaaccta acacaatgtg c 21

<210> SEQ ID NO 144
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 144

gcgccacctt tctcactca 19

<210> SEQ ID NO 145
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 145

cggtcccgtt cagttcgat 19

<210> SEQ ID NO 146
<211> LENGTH: 21
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<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 146

cctgagtaaa tactgccacc a 21

<210> SEQ ID NO 147
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 147

agaatgcgta atttctccct cg 22

<210> SEQ ID NO 148
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 148

tgtcttcagc atcaatttct tcac 24

<210> SEQ ID NO 149
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 149

ctgtaggctt gtttcatcat ca 22

<210> SEQ ID NO 150
<211> LENGTH: 193
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence from SBE IIb turgidum A/B genome (in Figure 3)

<400> SEQUENCE: 150

Arg Ile Leu Pro Pro Pro Gly Asn Gly Gln Gln Ile Tyr Glu Ile Asp
1 5 10 15

Pro Thr Leu Arg Asp Phe Lys Tyr His Leu Glu Tyr Arg Tyr Ser Leu
20 25 30

Tyr Arg Arg Ile Arg Ser Asp Ile Asp Glu His Lys Gly Gly Met Asp
35 40 45

Val Phe Ser Arg Gly Tyr Glu Lys Phe Gly Phe Val Arg Ser Ala Glu
50 55 60

Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala Asp Ser Ala Ala Leu
65 70 75 80

Val Gly Asp Phe Asn Asn Trp Asp Pro Asn Ala Asp His Met Ser Lys
85 90 95

Asn Asp Leu Gly Ile Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly
100 105 110

Ser Pro Pro Ile Pro His Gly Ser Arg Val Lys Val Arg Met Gly Thr
115 120 125

Pro Ser Gly Thr Lys Asp Ser Ile Pro Ala Trp Ile Lys Tyr Ser Val
130 135 140

Gln Thr Pro Gly Asp Ile Pro Tyr Asn Gly Ile Tyr Tyr Asp Pro Pro
145 150 155 160

Glu Glu Glu Lys Tyr Val Phe Lys His Pro Gln Pro Lys Arg Pro Lys
165 170 175

-continued

Ser Leu Arg Ile Tyr Glu Thr His Val Gly Met Ser Ser Pro Glu Pro
180 185 190

Lys

<210> SEQ ID NO 151
<211> LENGTH: 658
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Chara IIa B genome F1-13 0686 (in Figure 7)

<400> SEQUENCE: 151

cattcgtcaa ataataccct tgacggtttg aatggttttg atggcactga tacacattac 60

ttccacggtg gtccacgtgg ccatcattgg atgtgggatt ctcgtctgtt ccactatggg 120

agttgggaag tatgtagctg cgacttctgt caccatattt ggctaactgt tcctgtcaat 180

ctgttcttac acgtgtcaat attctattct tatacaggta ttaagattct tactgtcaaa 240

cgcgagatgg tggcttgatg aatataagtt tgatggattt cgatttgatg gggtgacctc 300

catgatgtat actcaccatg gattacaagt aagtcatcaa gtggtttcag taacttcttc 360

agggcactga aacaattgct atgcatcata acatgtatca tgatcagtac ttatgctacg 420

gagtctcaga tagttcccta gtatgcttgt acaattttac ctgacgagat catggaagat 480

tggaagtgat tgttattatt ttttcttcta agtttgcttc ttgttctaga tgacatttac 540

tgggaactat ggcgagtatt ttggatttgc cactgatgtt gatgcggtgg tatacttaat 600

gctggtcaac gatctaattc atggacttta tcctgatgct gtatccattg gtgaagaa 658

<210> SEQ ID NO 152
<211> LENGTH: 511
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Tasman0257 D genome (in Figure 8)

<400> SEQUENCE: 152

gcagaaaata tacgagattg acccaacact gaaagatttt cggagccatc ttgactaccg 60

gtaatgccta cccgctgctt tcgctcattt tgaattaagg tcctttcatc atgcaaattt 120

ggggaacatc aaagagacaa agactaggga ccaccatttc atacagatcc cctcgtggtc 180

tgagaatatg ctgggaagta aatgtataat tgatggctac aatttgctca aaattgcaat 240

acgaataact gtctccgatc attacaatta aagagtggca aactgatgaa atgtggtgga 300

tgggttatag attttacttt gctaattcct ctaccaaatt cctaggggggg aaatctacca 360

gttgggaaac ttagtttctt atctttgtgg ccttttttgtt ttggggaaaa cacattgcta 420

aattcgaatg attttgggta tacctcggtg gattcaacag atacagcgaa tacaagagaa 480

ttcgtgctgc tattgaccaa catgaaggtg g 511

<210> SEQ ID NO 153
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: CS_exon_3a D genome (in Figure 9)

<400> SEQUENCE: 153

gatacctgaa gatatcgagg agcaaacggc ggaagtgaac atgacagggg ggactgcaga 60

gaaacttcaa tcttcagaac cgactcaggg cattgtggaa acaatcactg atggtgtaac 120

-continued

caaaggagtt aaggaactag tcgtggggga gaaaccgcga gttgtcccaa aaccaggaga 180

tgggcagaaa atatacgaga ttgacccaac actgaaagat tttcggagcc atcttgacta 240

ccg 243

<210> SEQ ID NO 154
<211> LENGTH: 267
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of exon 1 region of SBEIIa gene (in Figure 10)

<400> SEQUENCE: 154

cacacgttgc tccccttct catcgcttct caattaatat ctccatcact cgggttccgc 60

gctgcatttc ggccggcggg ttgagtgaga tctgggccac tgaccgactc actcgctgcg 120

cggggatggc gacgttcgcg gtgtccggcg cgaccctcgg tgtggcgcgg cccgccagcg 180

ccggcggcgg actgctgcga tccggctcgg agcggagggg cggggtggac ttgccgtcgc 240

tgctcctcag gaagaaggac tcctctc 267

The claims defining the invention are as follows:

1. Wheat grain (*Triticum aestivum*) comprising an embryo, an endosperm, starch and a reduced level or activity of total SBEII protein, wherein the embryo comprises a loss of function mutation in alleles of endogenous genes of SBEIIa-A, SBEIIa-B, SBEIIa-D, SBEIIb-A, SBEIIb-B or SBEIIb-D, such that the level or activity of total SBEII protein in the grain is between 2% and 30% of the level or activity of total SBEII protein in a wild-type wheat grain, wherein

i) said alleles include 2, 4 or 6 SBEIIb alleles which are null alleles and 5 or 6 SBEIIa alleles which each comprise a loss of function mutation, wherein at least one of the 5 or 6 SBEIIa alleles which comprises a loss of function mutation comprises a loss of function point mutation;

ii) the grain has a germination rate of between about 70% and about 100% relative to the germination rate of a wild-type grain, and

iii) the starch of the grain has an amylose content of at least 50% (w/w) as determined by an iodometric method.

2. The wheat grain of claim **1**, wherein

a) the embryo of the grain is homozygous for alleles in each of 3 SBEIIa genes, each of the homozygous alleles comprising a loss of function mutation,

b) the number of null alleles of SBEIIa genes in the embryo is 2 or 4,

c) the embryo comprises two SBEIIb alleles which each comprise a partial loss of function mutation,

d) the grain comprises null alleles of the SBEIIa gene on the A genome, B genome, and D genomes; the A and B genomes; the A and D genomes; or the B and D genomes,

e) the grain comprises null alleles of the SBEIIb gene on the A genome; the A and B genomes; the A and D genomes; the B and D genomes; or all three of the A, B and D genomes,

f) the grain comprises only one SBEIIb protein which is detectable by Western blot analysis, wherein the one SBEIIb protein is encoded by the A genome, B genome or D genome,

g) the grain comprises only two SBEIIb proteins which are encoded by the A and B genomes, A and D genomes or B and D genomes,

h) the grain comprises a null mutation in an SBEIIa gene which is en amino acid substitution mutation,

i) each loss of function mutation in a SBEIIa allele is independently selected from the group consisting of a deletion mutation, an insertion mutation, a splice-site mutation, a premature translation termination mutation and a frameshift mutation, or

j) a combination of more than one of a) to i).

3. The grain of claim **1**, which comprises SBEIIa protein(s) which have starch branching activity when expressed in developing endosperm, each having an amount and/or starch branching enzyme activity of between 2% and 60% of the amount or activity of the SBEIIa protein expressed by the corresponding wild-type gene.

4. The grain of claim **1**, in which the level or activity of total SBEII protein in the grain is between 2% and 15% of the level or activity of total SBEII protein in the wild-type wheat grain.

5. The grain of claim **1**, in which the amount or activity of SBEIIa protein in the grain is between 2% and 15% of the amount or activity of SBEIIa protein in the wild-type wheat grain.

6. The wheat grain of claim **1** basing an amylose content in the starch of the grain of at least 60% (w/w) as determined by an iodometric method.

7. The grain of claim **1** which is non-transgenic or is free of any exogenous nucleic acid that encodes an RNA which reduces expression of an SBEIIa gene.

8. The grain of claim **1**, wherein the level or activity of total SBEII protein is determined by assaying the SBEII protein in grain while it is developing in a wheat plant or by assaying the level or activity of SBEII protein by immunological means.

9. The grain of claim **1**, wherein the starch of the grain is characterised by one or more of properties selected from the group consisting of:

(i) comprising at least 2% resistant starch,

(ii) comprising a reduced glycemic index (GI),

(iii) comprising a reduced level of amylopectin,

(iv) comprising distorted starch granules,

(v) having reduced starch granule birefringence,

(vi) reduced swelling volume,

(vii) modified chain length distribution and/or branching frequency,

(viii) delayed end of gelatinisation temperature and increased peak temperature,

(ix) reduced viscosity,

(x) increased molecular weight of amylopectin,

(xi) a modified percentage of starch crystallinity, and

(xii) a modified percentage of A-type or B-type starch, relative to starch of the wild-type grain.

10. The grain of claim **1** which is capable of producing a wheat plant which is male and female fertile.

11. A wheat plant (*Triticum aestivum*) which produces grain, the grain comprising an embryo, an endosperm, starch and a reduced level or activity of total SBEII protein, wherein the embryo comprises a loss of function mutation in alleles of endogenous genes of SBEIIa-A, SBEIIa-B, SBEIIa-D, SBEIIb-B or SBEIIb-D, such that the level or activity of total SBEII protein in the grain is between 2% and 30% of the level or activity of total SBEII protein in a wild-type wheat grain, wherein

i) said alleles include 2, 4 or 6 SBEIIb alleles which are null alleles and 5 or 6 SBEIIa alleles which each comprise a loss of function mutation, wherein at least one of the 5 or 6 SBEIIa alleles which comprises a loss of function mutation comprises a loss of function point mutation;

ii) the grain has a germination rate of between about 70% and about 100% relative to the germination rate of a wild-type grain;

iii) the starch of the grain has an amylose content of at least 50% 1w w) as determined by an iodometric method, and

iv) the wheat plant is male and female fertile.

12. A process for producing starch, comprising the steps of i) obtaining wheat grain according to claim **1**, and ii) extracting the starch from the grain, thereby producing the starch.

13. A process for producing bins of wheat grain comprising:

a) reaping wheat stalks comprising wheat grain according to claim **1**,

b) threshing and/or winnowing the stalks to separate the grain from the chaff, and

c) sifting and/or sorting the grain separated in step b), and loading the sifted and/or sorted grain into bins, thereby producing bins of wheat grain.

14. The wheat grain of claim **1**, wherein the grain comprises at least one SBEIIa protein which is produced in developing wheat endosperm and has starch branching enzyme activity.

15. The wheat grain of claim **1**, wherein five of the 5 or 6 SBEIIa alleles which each comprise a loss of function mutation each comprise a null mutation.

16. The wheat drain of claim **1**, wherein at least one of the 5 or 6 SBEIIa alleles which each comprise a loss of function mutation includes a mutation selected from the group consisting of an insertion mutation, a splice-site mutation, a premature translation termination mutation and a frameshift mutation.

17. The wheat grain of claim **1**, wherein the grain comprises only one or only two SBEIIb proteins which have starch branching enzyme activity when produced in developing endosperm.

18. The wheat grain of claim **1**, wherein the grain comprises a null mutation which is a deletion mutation in the A, B or D genome which deletes at least part of an SBEIIa gene and at least a part of an SBEIIb gene on that genome.

19. The wheat grain of claim **1**, wherein the grain comprises a null mutation which is a deletion mutation in the A, B or D genome which deletes the whole of the SBEIIa gene and/or the whole of the SBEIIb gene on that genome.

20. The wheat grain of claim **19**, wherein the grain comprises a null mutation which is a deletion mutation in the A genome which deletes the whole of the SBEIIa gene and/or the whole of the SBEIIb gene on that genome.

21. The wheat grain of claim **19**, wherein the grain comprises a null mutation which is a deletion mutation in the B genome which deletes the whole of the SBEIIa gene and/or the whole of the SBEIIb gene on that genome.

22. The wheat grain of claim **19**, wherein the grain comprises a null mutation which is a deletion mutation in the D genome which deletes the whole of the SBEIIa gene and/or the whole of the SBEIIa gene on that genome.

23. The wheat plant of claim **11**, wherein at least one of the 5 or 6 SBEIIa alleles which each comprise a loss of function mutation includes a mutation selected from the group consisting of an insertion mutation, a splice-site mutation, a premature translation termination mutation and a frameshift mutation.

24. A process for producing a food, comprising the steps of i) obtaining wheat grain according to claim **1**, and ii) processing the grain to produce a food ingredient, and iii) adding the food or drink ingredient to another food ingredient, thereby producing the food.

25. The process of claim **24**, wherein the food ingredient produced in step ii) is flour or wholemeal.

26. The wheat grain of claim **2**, wherein the embryo of the grain is homozygous for alleles in each of 3 SBEIIa genes, each of the homozygous alleles comprising a loss of function mutation.

27. The wheat grain of claim **2**, wherein the grain comprises null alleles of the SBEIIa gene on the A genome, B genome and D genomes; the A and B genomes; the A and D genomes; or the B and D genomes.

28. The wheat grain of claim **27**, wherein the grain comprises null alleles of the SBEIIa gene on the A genome, B genome, and D genomes.

29. The wheat grain of claim **2**, wherein the grain comprises null alleles of the SBEIIb gene on the A genome; the A and B genomes; the A and D geneses; the B and D genomes; or all three of the A, B and D genomes.

30. The wheat grain of claim **29**, wherein the grain comprises null alleles of the SBEIIb gene on the A genome.

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